(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 April 2003 (03.04.2003)

PCT

(10) International Publication Number
WO 03/027246 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US02/30412

(22) International Filing Date:
24 September 2002 (24.09.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/962,756 24 September 2001 (24.09.2001) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 09/962,756 (CIP)
Filed on 24 September 2001 (24.09.2001)

(71) Applicants *(for all designated States except US)*: **NOVO NORDISK A/S** [DK/DK]; Novo Allé, DK-2880 Bagsvaerd (DK). **DGI BIOTECHNOLOGIES** [US/US]; 40 Talmadge Road, Edison, NJ 08818-0424 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PILLUTLA, Renuka** [IN/US]; 10 Pope Road, Bridgewater, NJ 08807 (US). **DEDOVA, Olga** [—/US]; 68 Forrest Glen Drive, Highland Park, NJ 08904 (US). **BLUME, Arthur, J.** [US/US]; 11 Walden Drive, Annandale, NJ 08801 (US). **GOLDSTEIN, Neil, I.** [US/US]; 26 Kendal Avenue, Maplewood, NJ 07040 (US). **BRISSETTE, Renee** [US/US]; 20 Deer Trail Drive, Clarksburg, NJ 08510 (US). **WANG, Pinger** [—/US]; 3109 Birchwood Ct., No. Brunswick, NJ 08902 (US). **LIU, Hao** [—/US]; 42G Franklin Green S., 1 JFK Boulevard, Somerset, NJ 08873 (US). **HSIAO, Ku-chuan** [—/US]; 451 Milano Drive, Easton, PA 18040 (US). **LENNICK, Michael** [—/US]; 2 Hills Top Drive, Annandale, NJ 08801 (US). **FLETCHER, Paul** [—/US]; 424 Rivercrest Drive, Piscataway, NJ 08854 (US).

(74) Agents: **SONNENFELD, Kenneth, H.** et al.; Morgan & Finnegan L.L.P., 345 Park Avenue, New York, NY 10154 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/027246 A2

(54) Title: ANTAGONISTS

(57) Abstract: Peptide sequences capable of binding to insulin and/or insulin-like growth factor receptors with either agonist or antagonist activity and identified from various peptide libraries are disclosed. This invention also identifies at least two different binding sites, which are present on insulin and insulin-like growth factor receptors, and which selectively bind the peptides of this invention. As agonists, the peptides of this invention may be useful for development as therapeutics to supplement or replace endogenous peptide hormones. The antagonist peptides may also be developed as therapeutics.

INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

This application is a continuation-in-part of U.S. Application Serial No. 09/962,756 filed September 24, 2001, which is a continuation-in-part of U.S. Application Serial No. 09/538,038 filed March 29, 2000, which is a continuation-in-part of U.S. Application Serial No. 09/146,127, filed September 2, 1998, all of which are incorporated herein by reference in their entirety.

I. FIELD OF THE INVENTION

This invention relates to the field of hormone receptor activation or inhibition. More specifically, this invention relates to the identification of molecular structures, especially peptides, which are capable of acting at either the insulin or insulin-like growth factor receptors as agonists or antagonists. Also related to this invention is the field of molecular modeling whereby useful molecular models are derived from known structures.

II. BACKGROUND OF THE INVENTION

Insulin is a potent metabolic and growth promoting hormone that acts on cells to stimulate glucose, protein, and lipid metabolism, as well as RNA and DNA synthesis. A well-known effect of insulin is the regulation of glucose levels in the body. This effect occurs predominantly in liver, fat, and muscle tissue. In the liver, insulin stimulates glucose incorporation into glycogen and inhibits the production of glucose. In muscle and fat tissue, insulin stimulates glucose uptake, storage, and metabolism. Defects in glucose utilization are very common in the population, giving rise to diabetes.

Insulin initiates signal transduction in target cells by binding to a specific cell-surface receptor, the insulin receptor (IR). The binding leads to conformational changes in the extracellular domain of IR, which are

transmitted across the cell membrane and result in activation of the receptor's tyrosine kinase activity. This, in turn, leads to autophosphorylation of tyrosine kinase of IR, and the binding of soluble effector molecules that contain SH2 domains such as phophoinositol-3-kinase, Ras GTPase-activating protein, and phospholipase Cγ to IR (Lee and Pilch, 1994, *Am. J. Physiol.* **266**:C319-C334).

Insulin-like growth factor 1 (IGF-1) is a small, single-chain protein (MW = 7,500 Da) that is involved in many aspects of tissue growth and repair. It is similar in size, sequence, and structure to insulin, but has 100-1,000-fold lower affinity for IR (Mynarcik *et al.*, 1997, *J. Biol. Chem.* **272**:18650-18655). Although IGF-1 mRNA can be detected in many tissues, the majority of circulating IGF-1 is produced in the liver after stimulation by growth hormone (Butt *et al.*, 1999, *Immunol. Cell Biol.* **77**:256-262). Functionally, IGF-1 appears to act as a mitogen and as an anti-apoptotic factor for cells.

Recent studies have analyzed the role of endogenous IGF-1 in various disease states. Several reports have shown that IGF-1 promotes the growth of normal and cancerous prostate cells both *in vitro* and *in vivo* (Angelloz-Nicoud and Binoux, 1995, *Endocrinol.* **136**:5485-5492; Figueroa *et al.*, 1995, *J. Clin. Endocrinol. Metab.* **80**:3476-3482; Torring *et al.*, 1997, *J. Urol.* **158**:222-227). Elevated serum levels of IGF-1 have been shown to be associated with increased risks of prostate cancer, and may be an earlier predictor of onset than prostate-specific antigen (PSA; J.M. Chan *et al.*, 1998, *Science* **279**:563-566). Serum levels of free IGF-1 are regulated by the presence of IGF binding proteins (IGFBP), which bind to IGF-1 and prevent its interaction with the IGF-1R (reviewed in C.A. Conover, 1996, *Endocr. J.* **43S**:S43-S48; Rajaram et al., 1997, *Endocr. Rev.* **18**:801-831). PSA has been shown to be a protease that cleaves IGFBP-3, resulting in an increase of free IGF-1 in serum (P. Cohen *et al.*, 1992, *J. Clin. Endocrinol. Metab.* **75**:1046-1053; P. Cohen *et al.*, 1994, *J. Endocrinol.* **142**:407-415; H. Lilja, 1995, *Scand. J. Clin. Lab. Invest. Suppl.* **220**:47-56). Consistent with

this finding, men with higher levels of circulating IGF-1 and lower levels of IGFBP-3 were found to be at higher risk for developing colorectal cancer (J. Ma *et al.*, 1999, *J. Natl. Cancer Instit.* **91**:620-625.). Recent studies have also shown a connection between IGF-1 levels and ovarian cancer.

There also appears to be a relationship between high levels of IGF-1 and/or IGF-1R and breast cancer (L.C. Happerfield *et al.*, 1997, *J. Pathol.* **183**:412-417). A positive correlation was observed between circulating IGF-1 and breast cancer among pre-menopausal women (S.E. Hankinson *et al.*, 1998, *Lancet* **351**:1393-1396). A poor prognosis for breast cancer patients was correlated to the expression of IGF-1R positive and estrogen receptor (ER) negative cells (A.A. Butler *et al.*, 1998, *Cancer Res.* **58**:3021-3027). Recently, investigators have identified hybrid IGF-1R/IR receptors found in several breast cancer cell lines (G. Pandini *et al.*, 1999, *Clin. Cancer Res.* **5**:1935-1944; E.M. Bailyes *et al.*, 1997, *Biochem. J.* **327**(Pt 1):209-215; see below). The data has suggested that these hybrids behave as functional IGF-1Rs and may play a major role in IGF-1 signaling in breast cancer.

Clinical studies have also investigated the use of recombinant human IGF-1 in the treatment of several diseases, including type I diabetes (Carroll *et al.*, 1997, *Diabetes* **46**:1453-1458; Crowne *et al.*, 1998, *Metabolism* **47**:31-38), amyotropic lateral sclerosis (Lai *et al.*, 1997, *Neurology* **49**:1621-1630), and diabetic motor neuropathy (Apfel and Kessler, 1996, *CIBA Found. Symp.* **196**:98-108). Other potential therapeutic applications of IGF-1, such as osteoporosis (Canalis, 1997, *Bone* **21**:215-216), immune modulation (Clark, 1997, *Endocr. Rev.* **18**:157-179) and nephrotic syndrome (Feld and Hirshberg, 1996, *Pediatr. Nephrol.* **10**:355-358) are also under investigation. Clearly, IGF-1R activity is involved in many disease states, indicating that there are potential clinical applications for both IGF-1 agonists and antagonists.

Both insulin and IGF-1 are expressed as precursor proteins comprising, among other regions, contiguous A, B, and C peptide regions, with the C peptide being an intervening peptide connecting the A and B

peptides. A mature insulin molecule is composed of the A and B chains connected by disulfide bonds, where the connecting C peptide has been removed during post-translational processing. IGF-1 retains its smaller C-peptide as well as a small D extension at the C-terminal end of the A chain, making the mature IGF-1 slightly larger than insulin (Blakesley, 1996). The C region of human IGF-1 appears to be required for high affinity binding to IGF-1R (Pietrzkowski *et al.*, 1992, *Cancer Res.* **52**(23):6447-51). Specifically, tyrosine 31 located within this region appears to be essential for high affinity binding. Furthermore, deletion of the D domain of IGF-1 increased the affinity of the mutant IGF-1 for binding to the IR, while decreasing its affinity for the IGF-1R (Pietrzkowski *et al.*, 1992). A further distinction between the two hormones is that, unlike insulin, IGF-1 has very weak self-association and does not hexamerize (De Meyts, 1994).

IGF-1 and insulin competitively cross-react with IGF-1R and IR (L. Schäffer, 1994, *Eur. J. Biochem.* **221**:1127-1132). Yet, despite 45% overall amino acid identity, insulin and IGF-1 bind only weakly to each other's receptor. The affinity of each peptide for the non-cognate receptor is about 3 orders of magnitude lower than that for the cognate receptor (Mynarcik, *et al.*, 1997, *J. Biol. Chem.* **272**:18650-18655). The differences in binding affinities may be partly explained by the differences in amino acids and unique domains which contribute to unique tertiary structures of ligands (Blakesley *et al.*, 1996, *Cytokine Growth Factor Rev.* **7**(2):153-9).

IGF-1R and IR are related members of the tyrosine-kinase receptor superfamily of growth factor receptors. Another family member is insulin-related receptor (IRR), for which no natural ligand is known. Both IGF-1R and IR are comprised of two α and two β subunits which form a disulfide-linked heterotetramer (β-α-α-β). These receptors have an extracellular ligand binding domain, a single transmembrane domain, and a cytoplasmic domain displaying the tyrosine kinase activity. The extracellular domain is composed of the entire α subunits and a portion of the N-terminus of the β

subunits, while the intracellular portion of the β subunits contains the tyrosine kinase domain. In contrast to other tyrosine kinase receptors, IGF-1R, IR and IRR exist on the cell surface as disulphide-linked dimers and require domain rearrangements rather than receptor oligomerization for cell signaling (Adams *et al.*, 2000, *Cell. Mol. Life Sci.* **57**:1050-1093; Garrett *et al.*, 1998, *Nature* **394**:395-399; Frasca *et al.*, 1999, *Mol. Cell Biol.* **19**: 3278-3288; De Meyts *et al.*, 1994, *Hormone Res.* **42**:152-169). In addition, insulin and IGF-1 hemireceptors (comprising one α subunit and one β subunit) can heterodimerize to form IR/IGF-1R hybrids (M.A. Soos *et al.*, 1990, *Biochem. J.* **270**:383-390; J. Kasua *et al.*, 1993, *Biochemistry* **32**:13531-13536; B.L. Seely *et al.*, 1995, *Endocrinology* **136**:1635-1641).

In many cells, IR/IGF-1R hybrids are the most common receptor subtype (Bailyes *et al.*, 1997, *Biochem. J.* **327**(pt.1):209-215). The proportion of total IGF-1R assembled into hybrids varies between 40% and 60% in human tissues (M. Federici *et al.*, 1997, *Mol. Cell. Endocrin.* **129**(2):121-6). IR/IGF-1R hybrids are also overproduced in human cancer cells as a result of overexpression of IR and IGF-1R (Pandini *et al.*, 1999, *Clin. Cancer Res.* **5**:1935-1944; A. Belfiore *et al.*, 1999, *Biochemie*, **81**(4):403-7; V. Papa *et al.*, 1990, *J. Clin. Invest.* 86:1503-1510; V. Papa *et al.*, 1993, *Cancer Res.* **53**:3736-3740). In particular, increased levels of IR/IGF-1R hybrids have been observed in breast cancer cell lines and breast cancer tissue specimens (Pandini *et al.*, 1999, *Clin. Cancer Res.* **5**:1935-1944). Similarly, high levels of IR/IGF-1R hybrids have been observed in thyroid cancer specimens and cell lines (A. Belfiore *et al.*, 1999, *Biochemie*, **81**(4):403-7). Functional studies have indicated that IR/IGF-1R hybrids are predominantly activated by IGF-1 (M.A. Soos *et al.*, 1993, *Biochem. J.* **290**(pt.2):419-426; A.L. Frattali *et al.*, 1993, *J. Biol. Chem.* **268**:7393-7400). Accordingly, it has been postulated that IR/IGF-1R hybrids provide additional binding sites for IGF-1, and thereby increase cell sensitivity to this factor (Bailyes *et al.*, 1997, *Biochem. J.* **327**(pt.1):209-215;

Pandini *et al.*, 1999, *Clin. Cancer Res.* **5**:1935-1944; A. Belfiore *et al.*, 1999, *Biochemie*, **81**(4):403-7).

IR is a glycoprotein having molecular weight of 350-400 kDa (depending of the level of glycosylation). It is synthesized as a single polypeptide chain and proteolytically cleaved to yield a disulfide-linked monomer α-β insulin receptor. Two α-β monomers are linked by disulfide bonds between the α-subunits to form a dimeric form of the receptor (β-α-α-β-type configuration). The α subunit is comprised of 723 amino acids, and it can be divided into two large homologous domains, L1 (amino acids 1-155) and L2 (amino acids 313-468), separated by a cysteine-rich region (amino acids 156-312) (Ward *et al.*, 1995, *Prot. Struct. Funct. Genet.* **22**:141-153). Many determinants of insulin binding seem to reside in the α-subunit. The β-subunit of IR has 620 amino acid residues and three domains: extracellular, transmembrane, and cytosolic. The extracellular domain is linked by disulfide bridges to the α-subunit. The cytosolic domain includes the tyrosine kinase domain, the three-dimensional structure of which has been solved (Hubbard *et al.*, 1994, *Nature* **372**:746-754). A unique feature of IR is that it is dimeric in the absence of ligand.

To aid in drug discovery efforts, a soluble form of a membrane-bound receptor was constructed by replacing the transmembrane domain and the intracellular domain of IR with constant domains from immunoglobulin Fc or λ subunits (Bass *et al.*, 1996, *J. Biol. Chem.* **271**:19367-19375). The recombinant gene was expressed in human embryonic kidney 293 cells. The expressed protein was a fully processed heterotetramer and the ability to bind insulin was similar to that of the full-length holoreceptor.

IGF-1R is synthesized as a 180 kDa precursor which is glycosylated, dimerized and proteolytically processed to yield mature receptor (T.E. Adams *et al.*, 2000, *Cell. Mol. Life Sci.*, **57**:1050-1093, 2000). The mature receptor/complex consists of two extracellular α-subunits and two transmembrane β-subunits having tyrosine kinase activity. IGF-1R is

expressed in almost all normal adult tissue except for liver, which is itself the major site of IGF-1 production (Butt *et al.*, 1999, *Immunol. Cell Biol.* **77**:256-262). A variety of signaling pathways are activated following binding of IGF-1 to the IGF-1R, including Src and ras, as well as downstream pathways, such as the MAP kinase cascade and the PI3K/AKT axis (Chow *et al.*, 1998, *J. Biol. Chem.* **273**:4672-4680).

The sequence of IR is highly homologous to the sequence of IGF-1R, indicating that the three-dimensional structures of both receptors may be similar. The α-subunits, which contain the ligand binding region of IR and IGF-1R, exhibit between 47-67% overall amino acid identity. Three general domains, termed L1, cysteine-rich, and L2, have been reported for both receptors from sequence analysis of the α subunits. The cysteine residues in the cysteine-rich region are highly conserved between the two receptors; however, the cysteine-rich regions share only 48% overall amino acid identity. Notably, the crystal structure of the first three domains of IGF-1R has been determined (Garrett *et al.*, 1998, *Nature* **394**:395-399). The L domains consist of a single-stranded right-handed β-helix (a helical arrangement of β-strands), while the cysteine-rich region is composed of eight disulfide-bonded modules.

While similar in structure, IGF-1R and IR serve different physiological functions. IR is primarily involved in metabolic functions whereas IGF-1R mediates growth and differentiation. Consistent with this, ablation of IGF-1 (i.e., in IGF-1 knock-out mice) results in embryonic growth deficiency, impaired postnatal growth, and infertility. In addition, IGF-1R knock-out mice were only 45% of normal size and died of respiratory failure at birth (Liu *et al.*, 1993, *Cell* **75**:59-72). However, both insulin and IGF-1 can induce both mitogenic and metabolic effects. Whether each ligand elicits both activities via its own receptor, or whether insulin exerts its mitogenic effects through its weak affinity binding to IGF-1R, and IGF-1 its metabolic

effects through IR, remains controversial (De Meyts, 1994, *Horm. Res.* **42**:152-169).

Also, despite the similarities observed between these two receptors, the role of the domains in specific ligand binding are distinct. Through chimeric receptor studies, (domain swapping of the IR and IGF-1R α-subunits), researchers have reported that the sites of interaction of the ligands with their specific receptors differ (T. Kjeldsen *et al.*, 1991, *Proc. Natl. Acad. Sci. USA* **88**:4404-4408; A.S. Andersen *et al.*, 1992, *J. Biol. Chem.* **267**:13681-13686). For example, the cysteine-rich domain of the IGF-1R was determined to be essential for high-affinity IGF binding, but not insulin binding. When amino acids 191-290 of IGF-1R region was introduced into the corresponding region of the IR (amino acids 198-300), the modified IR bound both IGF-1 and insulin with high affinity. Conversely, when the corresponding region of the IR was introduced into the IGF-1R, the modified IGF-1R bound to IR but not IGF-1.

A further distinction between the binding regions of the IR and IGF-1R is their differing dependence on the N-terminal and C-terminal regions. Both the N-terminal and C-terminal regions (located within the putative L1 and L2 domains) of the IR are important for high-affinity insulin binding but appear to have little effect on IGF-1 binding for either IR or IGF-1R. Replacing residues in the N-terminus of IGF-1R (amino acids 1-62) with the corresponding residues of IR (amino acids 1-68) confers insulin-binding ability on IGF-1R. Within this region, residues Phe-39, Arg-41 and Pro-42 are reported as major contributors to the interaction with insulin (Williams *et al.*, 1995). When these residues are introduced into the equivalent site of IGF-1R, the affinity for insulin is markedly increased, whereas, substitution of these residues by alanine in IR results in markedly decreased insulin affinity. Similarly, the region between amino acids 704-717 of the C-terminus of IR has been shown to play a major role in insulin specificity. Substitution of these residues with alanine also disrupts insulin binding

(Mynarcik *et al.*, 1996, *J. Biol. Chem.* **271**(5):2439-42; C. Kristensen *et al.*, 1999, *J. Biol. Chem.* **274**(52):37351-37356).

Alanine scans of IR and IGF-1R suggest that insulin and IGF-1 may use some common contacts to bind to IGF-1R but that those contacts differ from those that insulin utilizes to bind to IR (Mynarcik *et al.*, 1997). Hence, the data in the literature has led one commentator to state that even though "the binding interfaces for insulin and IGF-1 on their respective receptors may be homologous within this interface the side chains which make actual contact and determine specificity may be quite different between the two ligand-receptor systems" (De Meyts, 1994).

Based on data for binding of insulin and insulin analogs to various insulin receptor constructs, a binding model has been proposed. This model shows insulin receptor with two insulin binding sites that are positioned on two different surfaces of the receptor molecule, such that each alpha-subunit is involved in insulin binding. In this way, activation of the insulin receptor is believed to involve cross-connection of the alpha-subunits by insulin. A similar mechanism may operate for IGF-1R, but one of the receptor binding interactions appears to be different (Schäffer, 1994, *Eur. J. Biochem.* **221**:1127-1132).

The identification of molecular structures having a high degree of specificity for one or the other receptor is important to developing efficacious and safe therapeutics. For example, a molecule developed as an insulin agonist should have little or no IGF-1 activity in order to avoid the mitogenic activity of IGF-1 and a potential for facilitating neoplastic growth. It is therefore important to determine whether insulin and IGF-1 share common three-dimensional structures but which have sufficient differences to confer selectivity for their respective receptors. Similarly, it would be desirable to identify other molecular structures that mimic the active binding regions of insulin and/or IGF-1 and which impart selective agonist or antagonist activity.

Although certain proteins are important drugs, their use as therapeutics presents several difficult problems, including the high cost of production and formulation, administration usually via injection and limited stability in the bloodstream. Therefore, replacing proteins, including insulin or IGF-1, with small molecular weight drugs has received much attention. However, to date, none of these efforts has resulted in finding an effective drug replacement.

Peptides mimicking functions of protein hormones have been previously reported. Yanofsky *et al.* (1996, *Proc. Natl. Acad. Sci. USA* 93:7381-7386) reported the isolation of a monomer antagonistic to IL-1 with nanomolar affinity for the IL-1 receptor. This effort required construction and use of many phage displayed peptide libraries and sophisticated phage-panning procedures.

Wrighton *et al.* (1996, *Science* **273**:458-464) and Livnah *et al.* (1996, *Science* **273**:464-471) reported dimer peptides that bind to the erythropoietin (EPO) receptor with full agonistic activity *in vivo*. These peptides are cyclical and have intra-peptide disulfide bonds; like the IL-1 receptor antagonist, they show no significant sequence identity to the natural ligand. Importantly, X-ray crystallography revealed that it was the spontaneous formation of non-covalent peptide homodimer peptides that enabled the dimerization two EPO receptors.

WO 96/04557 reported the identification of peptides and antibodies that bound to active sites of biological targets, which were subsequently used in competition assays to identify small molecules that acted as agonist or antagonists at the biological targets. Renchler *et al.* (1994, *Proc. Natl. Acad. Sci. USA* **91**:3623-3627) reported synthetic peptide ligands of the antigen binding receptor that induced programmed cell death in human B-cell lymphoma.

Most recently, Cwirla *et al.* (1997, *Science* **276**:1696-1698) reported the identification of two families of peptides that bound to the human thrombopoietin (TPO) receptor and were competed by the binding of the

natural TPO ligand. The peptide with the highest affinity, when dimerized by chemical means proved to be as potent an *in vivo* agonist as TPO, the natural ligand.

III. SUMMARY OF THE INVENTION

This invention relates to the identification of amino acid sequences that specifically recognize sites involved in IR or IGF-1R activation. Specific amino acid sequences are identified and their agonist or antagonist activity at IR and/or IGF-1R has been determined. Such sequences may be developed as potential therapeutics or as lead compounds to develop other more efficacious ones. In addition, these sequences may be used in high-throughput screens to identify and provide information on small molecules that bind at these sites and mimic or antagonize the functions of insulin or IGF-1. Furthermore, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which can be used to identify sequence variants that increase or otherwise modulate the binding and/or activity of the original peptide at IR or IGF-1R. The peptide sequences of the invention can also be combined to make dimer or other multimeric peptides, which can be used for screening, diagnostic, and thereapeutic applications as described herein.

In one aspect of this invention, large numbers of peptides have been screened for their IR and IGF-1R binding and activity characteristics. Analysis of their amino acid sequences has identified certain consensus sequences which may be used themselves or as core sequences in larger amino acid sequences conferring upon them agonist or antagonist activity. Several generic amino acid sequences are disclosed which bind IR and/or IGF-1R with varying degrees of agonist or antagonist activity depending on the specific sequence of the various peptides identified within each motif group. Also provided are amino or carboxyl terminal extensions capable of modifying the affinity and/or pharmacological activity of the consensus sequences when part of a larger amino acid sequence. Further provided

are peptides containing more than one consensus sequence (e.g., dimer peptides).

The amino acid sequences of this invention which bind IR and/or IGF-1R include:

a. $X_1\ X_2\ X_3\ X_4\ X_5$ wherein X_1, X_2, X_4 and X_5 are aromatic amino acids, and X_3 is any polar amino acid (Formula 1; Group 1; A6 motif);

b. $X_6\ X_7\ X_8\ X_9\ X_{10}\ X_{11}\ X_{12}\ X_{13}$ wherein X_6 and X_7 are aromatic amino acids, X_8, X_9, X_{11} and X_{12} are any amino acid, and X_{10} and X_{13} are hydrophobic amino acids (Formula 2; Group 3; B6 motif);

c. $X_{14}\ X_{15}\ X_{16}\ X_{17}\ X_{18}\ X_{19}\ X_{20}\ X_{21}$ wherein X_{14}, and X_{17} are hydrophobic amino acids, X_{15}, X_{16}, X_{18} and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids (Formula 3; reverse B6; revB6).

d. $X_{22}\ X_{23}\ X_{24}\ X_{25}\ X_{26}\ X_{27}\ X_{28}\ X_{29}\ X_{30}\ X_{31}\ X_{32}\ X_{33}\ X_{34}\ X_{35}\ X_{36}\ X_{37}\ X_{38}\ X_{39}\ X_{40}\ X_{41}$ wherein X_{22}, X_{25}, X_{28}, X_{29}, X_{30}, X_{33}, X_{34}, X_{35}, X_{36}, X_{37}, X_{38}, X_{40}, and X_{41} are any amino acid, X_{35} and X_{37} may be any amino acid for binding to IR, whereas X_{35} is preferably a hydrophobic amino acid and X_{37} is preferably glycine for binding to IGF-1R and possess agonist or antagonist activity. X_{23} and X_{26} are hydrophobic amino acids. This sequence further comprises at least two cysteine residues, preferably at X_{25} and X_{40} X_{31} and X_{32} are small amino acids (Formula 4; Group 7; E8 motif).

e. $X_{42}\ X_{43}\ X_{44}\ X_{45}\ X_{46}\ X_{47}\ X_{48}\ X_{49}\ X_{50}\ X_{51}\ X_{52}\ X_{53}\ X_{54}\ X_{55}\ X_{56}\ X_{57}\ X_{58}\ X_{59}\ X_{60}\ X_{61}$ wherein X_{42}, X_{43}, X_{44}, X_{45}, X_{53}, X_{55}, X_{56}, X_{58}, X_{60} and X_{61} may be any amino acid, X_{43}, X_{46}, X_{49}, X_{50}, X_{54} are hydrophobic amino acids, X_{47} and X_{59} are preferably cysteines, X_{48} is a polar amino acid, and X_{51}, X_{52} and X_{57} are small amino acids (Formula 5; mini F8 motif).

f. $X_{62}\ X_{63}\ X_{64}\ X_{65}\ X_{66}\ X_{67}\ X_{68}\ X_{69}\ X_{70}\ X_{71}\ X_{72}\ X_{73}\ X_{74}\ X_{75}\ X_{76}\ X_{77}\ X_{78}\ X_{79}\ X_{80}\ X_{81}$ wherein X_{62}, X_{65}, X_{68}, X_{69}, X_{71}, X_{73}, X_{76}, X_{77}, X_{78}, X_{80}, and X_{81} may be any amino acid; X_{63}, X_{70}, X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid, X_{67} and X_{75} are aromatic amino acids and X_{72} and X_{79} are preferably cysteines capable of forming a loop (Formula 6; Group 2; D8 motif).

g. H X_{82} X_{83} X_{84} X_{85} X_{86} X_{87} X_{88} X_{89} X_{90} X_{91} X_{92} wherein X_{82} is proline or alanine, X_{83} is a small amino acid, X_{84} is selected from leucine, serine or threonine, X_{85} is a polar amino acid, X_{86}, X_{88}, X_{89} and X_{90} are any amino acid, and X_{87}, X_{91} and X_{92} are an aliphatic amino acid (Formula 7).

h. X_{104} X_{105} X_{106} X_{107} X_{108} X_{109} X_{110} X_{111} X_{112} X_{113} X_{114} wherein at least one of the amino acids of X_{106} through X_{111}, and preferably two, are tryptophan separated by three amino acids, and wherein at least one of X_{104}, X_{105} and X_{106} and at least one of X_{112}, X_{113} and X_{114} are cysteine (Formula 8); and

i. an amino acid sequence comprising the sequence JBA5: DYKDLCQSWGVRIGWLAGLCPKK (SEQ ID NO:1541) or JBA5 minus FLAG® tag and terminal lysines: LCQSWGVRIGWLAGLCP (SEQ ID NO:1542) (Formula 9).

j. W X_{123} G Y X_{124} W X_{125} X_{126} (SEQ ID NO:1543) wherein X_{123} is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; X_{124} is any amino acid, but preferably a charged or aromatic amino acid; X_{125} is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. X_{126} is any amino acid, but preferably a small amino acid (Formula 10; Group 6 motif).

In one embodiment, peptides comprising a preferred amino acid sequence FYX_3 WF (SEQ ID NO:1544) (Formula 1; Group 1; A6 motif) have been identified which competitively bind to sites on IR. Surprisingly, peptides comprising an amino acid sequence FYX_3 WF (SEQ ID NO:1544) can possess agonist or antagonist activity at IR or IGF-1R.

This invention also identifies at least two distinct binding sites on IR and IGF-1R (Site 1 and Site 2) based on the differing ability of certain of the peptides to compete with one another and ligand for binding to IR or IGF-1R. Accordingly, this invention provides amino acid sequences that bind specifically to one or both sites of IR or IGF-1R. Furthermore, specific amino acid sequences are provided which have agonist or antagonist

characteristics based on their ability to bind to the specific sites of IR or IGF-1R.

In another embodiment of this invention, amino acid sequences which bind to one or more sites of IR or IGF-1R (e.g., Site 1 or Site 2) are covalently linked together to form multivalent ligands. These multivalent ligands are capable of forming complexes with a plurality of IR or IGF-1R. Either the same or different amino acid sequences are covalently bound together to form homo- or heterocomplexes.

In various aspects of the invention, monomer subunits are covalently linked at their N-termini or C-termini to form N-N, C-C, N-C, or C-N linked dimer peptides. In one example, dimer peptides are used to form receptor complexes bound through the same corresponding sites, e.g., Site 1-Site 1 or Site 2-Site 2 dimers. Alternatively, heterodimer peptides are used to bind to different sites on one receptor or to cause receptor complexing through different sites, e.g., Site 1-Site 2 or Site 2-Site 1 dimers. In one novel aspect of the invention, Site 2-Site 1 dimers find use as insulin agonists, while certain Site 1-Site 2 dimers find use as insulin antagonists.

In various embodiments, insulin agonists comprise Site 1-Site 1 dimer peptide sequences S325, S332, S333, S335, S337, S353, S374-S376, S378, S379, S381, S414, S415, and S418; whereas other insulin agonists comprise Site 2-Site 1 dimer peptide sequences S455, S457, S458, S467, S468, S471, S499, S510, S518, S519, and S520, as described herein below. In one preferred embodiment, an insulin agonist comprises the sequence of the S519 dimer peptide, which shows insulin-like activity in both *in vitro* and *in vivo* assays.

The present invention also provides assays for identifying compounds that mimic the binding characteristics of insulin or IGF-1. Such compounds may act as antagonists or agonists of insulin or IGF-1 function in cell based assays.

This invention further provides kits for identifying compounds that bind to IR and/or IGF-1R. Also provided are therapeutic compounds that bind the insulin receptor or the IGF-1 receptor.

Other embodiments of this invention are the nucleic acid sequences encoding the amino acid sequences of the invention. Also within the scope of this invention are vectors containing the nucleic acids and host cells which express the nucleic acids encoding the amino acid sequences which bind at IR and/or IGF-1R and possess agonist or antagonist activity.

This invention also provides amino acid sequences that bind to active sites of IR and/or IGF-1R and to identify structural criteria for conferring agonist or antagonist activity at IR or IGF-1R.

This invention further provides specific amino acid sequences that possess agonist, partial agonist, or antagonist activity at either IR or IGF-1R. Such amino acid sequences are potentially useful as therapeutics themselves or may be used to identify other molecules, especially small organic molecules, which possess agonist or antagonist activity at IR or IGF-1R.

In addition, the present invention provides structural information derived from the amino acid sequences of this invention, which may be used to construct other molecules possessing the desired activity at the relevant IR binding site.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1O; 2A-2E; 3A-3E; 4A-4I; 43A-43B, 44A-44B: Amino acid sequences identified by panning peptide libraries against IGF-1R and/or IR. The amino acids are represented by their one-letter abbreviation. The ratios over background are determined by dividing the signal at 405 nm (E-Tag, IGF-1R, or IR) by the signal at 405 nm for non-fat milk. The IGF-1R/IR Ratio Comparison is determined by dividing the ratio of IGF-1R by the ratio of IR. The IR/IGF-1R Ratio Comparison is determined by dividing the ratio of IR by the ratio of IGF-1R. HIT indicates binder; CAND indicates binder candidate;

LDH indicates binding to lactate dehydrogenase (negative control); Sp/Irr indicates the ratio of specific binding over non-specific binding.

The design of each library is shown in the first line in bold. In the design, symbol 'X' indicates a random position, an underlined amino acid indicates a doped position at the nucleotide level, and other positions are held constant. Additional abbreviations in the B6H library are: 'O' indicates an NGY codon where Y is C or T; 'J' indicates an RHR codon where R is A or G, and H is A, C, or T; and 'U' indicates an VVY codon where V is A, C, or G, and Y is C or T. The 'h' in the 20E2 libraries indicates an NTN codon.

Symbols in the listed sequences include: Q indicates a position corresponding to a TAG stop codon; # indicates a position corresponding to a TAA stop codon; * indicates a position corresponding to a TGA stop codon; and ? indicates an unknown amino acid. It is believed that a W replaces the TGA stop codon when expressed. The Q residues represent translation read-through at TAG stop codons. Except for the 20C and A6L libraries, all libraries are designed with the short FLAG® epitope DYKD (SEQ ID NO:1545; Hopp *et al.*, 1988, *Bio/Technology* **6**:1205-1210) at the N-terminus of the listed sequence and AAAGAP (SEQ ID NO:1546) at the C-terminus. The 20C and A6L libraries have the full length FLAG® epitope DYKDDDDK (SEQ ID NO:1547).

Figure 1A: Formula 1 motif peptide sequences obtained from a random 40mer library panned against IR (SEQ ID NOS:1-3).

Figure 1B: Formula 1 motif peptide sequence obtained from a random 40mer library panned against IGF-1R (SEQ ID NOS:4-6).

Figure 1C: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IR (SEQ ID NOS:7-29).

Figure 1D: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IGF-1R (SEQ ID NOS:30-33).

Figure 1E: Formula 1 motif peptide sequences obtained from a 21mer library constructed to contain X_{1-10}NFYDWFVX_{18-21} (SEQ ID NO:34; also referred to as "A6S") panned against IR (SEQ ID NOS:35-98).

Figure 1F: Formula 1 motif peptide sequences obtained from a 21mer library constructed to contain X_{1-10}NFYDWFVX_{18-21} (SEQ ID NO:34; also referred to as "A6S") panned against IGF-1R (SEQ ID NOS:99-166).

Figure 1G: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L" (SEQ ID NO:167)) panned against IR (SEQ ID NOS:168-216).

Figure 1H: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L" (SEQ ID NO:167)) panned against IGF-1R (SEQ ID NOS:217-244).

Figure 1I: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (SEQ ID NO:245) (as indicated) panned against IR (SEQ ID NOS:246-305).

Figure 1J: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (SEQ ID NO:245) (as indicated) panned against IGF-1R (SEQ ID NOS:306-342).

Figure 1K: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6}FHENFYDWFVRQVSX_{21-26} (SEQ ID NO:343; H2C-A) panned against IR (SEQ ID NOS:344-430).

Figure 1L: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6}FHENFYDWFVRQVSX_{21-26} (SEQ ID NO:343; H2C-A) panned against IGF-1R (SEQ ID NOS:431-467).

Figure 1M: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6}FHXXFYXWFX_{16-21} (SEQ ID NO:468; H2C-B) and panned against IR (SEQ ID NOS:469-575).

Figure 1N: Formula 1 motif peptide sequences obtained from a library constructed using the sequence X_{1-6}FHXXFYXWFX_{16-21} (SEQ ID NO:468; H2C-B) and panned against IGF-1R (SEQ ID NOS:576-657).

Figure 1O: Formula 1 motif peptide sequences obtained from other libraries panned against IR (SEQ ID NOS:658-712).

Figure 2A: Formula 4 motif peptide sequences identified from a random 20mer library panned against IR (SEQ ID NO:713).

Figure 2B: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide (SEQ ID NO:713) as indicated (15% dope; referred to as "F815") panned against IR (SEQ ID NOS:714-796).

Figure 2C: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide (SEQ ID NO:713) as indicated (15% dope; referred to as "F815") panned against IGF-1R (SEQ ID NOS:797-811).

Figure 2D: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide (SEQ ID NO:713)as indicated (20% dope; referred to as "F820") panned against IR (SEQ ID NOS:812-861).

Figure 2E: Formula 4 motif peptide sequences identified from other libraries panned against IR (SEQ ID NOS:862-925).

Figure 3A: Formula 6 motif peptide sequences identified from a random 20mer library and panned against IR (SEQ ID NOS:926-928).

Figure 3B: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide (SEQ ID NO:929) as indicated (15% dope; referred to as "D815") panned against IR (SEQ ID NOS:930-967).

Figure 3C: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide (SEQ ID NO:929) as indicated (20% dope; referred to as "D820") panned against IR (SEQ ID NOS:968-1010).

Figure 3D: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide (SEQ ID NO:929)

as indicated (20% dope; referred to as "D820") panned against IGF-1R (SEQ ID NOS:1011-1059).

Figure 3E: Formula 6 motif peptide sequences identified from other libraries panned against IR (SEQ ID NOS:1060-1061).

Figure 4A: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IGF-1R (SEQ ID NOS:1062-1077).

Figure 4B: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IR (SEQ ID NOS:1078-1082).

Figure 4C: Miscellaneous peptide sequences identified from a random 20mer library panned against IR (SEQ ID NOS:1083-1086).

Figure 4D: Miscellaneous peptide sequences identified from a random 40mer library panned against IR (SEQ ID NOS:1087-1088).

Figure 4E: Miscellaneous peptide sequences identified from a random 20mer library panned against IGF-1R (SEQ ID NOS:1089-1092).

Figure 4F: Miscellaneous peptide sequences identified from an X_{1-4} C X_{6-20} library and panned against IGF-1R (SEQ ID NOS:1093-1113).

Figure 4G: Miscellaneous peptide sequences identified from a library constructed to contain variations of the F8 peptide(SEQ ID NO:1114) as indicated (F815) panned against IGF-1R (SEQ ID NOS:1115-1118).

Figure 4H: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide(SEQ ID NO:1119) as indicated (referred to as "NNKH") panned against IR (SEQ ID NOS:1120-1142).

Figure 4I: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide(SEQ ID NO:1119) as indicated (referred to as "NNKH") panned against IGF-1R (SEQ ID NOS:1143-1154).

Figure 5A: Summary of specific representative amino acid sequences from Formulas 1, 4, 6, and 10 (SEQ ID NOS:1155-1180).

Figure 5B: Summary of specific representative amino acid sequences from Formulas 1, 4, 6, and 10 (SEQ ID NOS:1181-1220).

Figure 6: Illustration of 2 binding site domains on IR based on competition data.

Figure 7: Schematic illustration of potential binding schemes to the multiple binding sites on IR.

Figure 8: Biopanning results and sequence alignments of Group 1 of IR-binding peptides (SEQ ID NOS:1221-1243). The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or IGF-1R receptor. Absorbance signals are indicated by: ++++, >30X over background; +++, 15-30X; ++, 5-15X; +, 2-5X; and 0, <2X.

Figures 9A-9B: Biopanning results and sequence alignments of Groups 2, 6, and 7 of IR-binding peptides (SEQ ID NOS:1244-1261). The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or IGF-1R receptor. Absorbance signals are indicated by: ++++, >30X over background; +++, 15-30X; ++, 5-15X; +, 2-5X; and 0, <2X.

Figures 10A-10C: Insulin competition data determined for various monomer and dimer peptides. Figure 10A shows the competition curve. Figure 10B shows the symbol key for the peptides. Figure 10C shows the description of the peptides.

Figures 11A-11D: Insulin competition data determined for various monomer and dimer peptides. Figure 11A shows the competition curve. Figure 11B shows the symbol key for the peptides. Figure 11C shows the description of the peptides. Figure 11D shows IR binding affinity for the peptides.

Figures 12A-12D: Results of free fat cell assays for truncated synthetic RP9 monomer peptides, S390 and S394. Figure 12A shows the results for peptide S390. Figure 12B shows the results for peptide S394. Figure 12C shows the amino acid sequence of peptides S390 and S394 (SEQ ID NOS:1794 and 1788, respectively in order of appearance). Figure 12D shows the results for full-length RP9 peptide.

Figures 13A-13C: Results of free fat cell assays for truncated synthetic RP9 dimer peptides, S415 and S417. Figure 13A shows the results for peptide S415. Figure 13B shows the results for peptide S417. Figure 13C shows the amino acid sequence of peptides S415 and S417 (SEQ ID NOS:1795-1796).

Figures 14A-14C: Results of free fat cell assays for RP9 homodimer peptides, 521 and 535. Figure 14A shows the results for peptide 521. Figure 14B shows the results for peptide 535. Figure 14C shows the amino acid sequence of peptides 521 and 535.

Figures 15A-15C: Results of free fat cell assays for RP9-D8 heterodimer peptides, 537 and 538. Figure 15A shows the results for peptide 537. Figure 15B shows the results for peptide 538. Figure 15C shows the amino acid sequence of peptides 537 and 538.

Figures 16A-16C: Results of free fat cell assays for RP9-D8 heterodimer peptides 537 and 538. Figure 16A shows the results for peptide 537. Figure 16B shows the results for peptide 538. Figure 16C shows the amino acid sequence of peptides 537 and 538.

Figures 17A-17B: Results of free fat cell assays for D8-RP9 heterodimer peptide, 539. Figure 17A shows the results for peptide 539. Figure 17B shows the amino acid sequence of peptide 539.

Figures 18A-18D: Results of free fat cell assays for Site 1/Site 2 dimer peptides with constituent monomer peptides with Site 1-Site 2 C-N (Figure 18A), Site 1-Site 2, N-N (Figure 18B), Site 1-Site 2, C-C (Figure 18C), and Site 2-Site 1, C-N (Figure 18D) orientations and linkages, respectively.

Figures 19A-19B: Results of human insulin receptor kinase assays for various monomer and dimer peptides. Figure 19A shows the substrate phosphorylation curve. Figure 19B shows the EC_{50} values.

Figures 20A-20B: Results of human insulin receptor kinase assays for Site 1-Site 2 and Site 2-Site 1 dimer peptides. Figure 20A shows the substrate phosphorylation curve. Figure 20B shows the EC_{50} values.

Figures 21A-21B: Results of human insulin receptor kinase assays for Site 1-Site 2 and Site 2-Site 1 peptides. Figure 21A shows the substrate phosphorylation curve. Figure 21B shows the EC_{50} values.

Figures 22A-22B: Results of time-resolved fluorescence resonance transfer assays for assessing the ability of various monomer and dimer peptides to compete with biotinylated RP9 monomer peptide for binding to soluble human insulin receptor-immunoglobulin heavy chain chimera. Figure 22A shows the binding curve. Figure 22B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

Figures 23A-23C: Results of time-resolved fluorescence resonance transfer assays indicating the ability of various monomer and dimer peptide to compete with biotinylated S175 monomer peptide or biotinylated RP9 monomer peptide for binding to soluble human insulin receptor-immunoglobulin heavy chain chimera. Figures 23A-23B show the binding curves. Figure 23C shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

Figures 24A-24B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptide to compete with fluoroscein labeled RP9 monomer peptide for binding to soluble human insulin receptor ectodomain. Figure 24A shows the binding curve. Figure 24B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560 and 2001-2002, respectively in order of appearance).

Figures 25A-25B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptides to compete with fluoroscein labeled RP9 monomer peptide for binding to soluble human insulin mini-receptor. Figure 25A shows the binding curve. Figure 25B

shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

Figures 26A-26B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptides to compete with fluorescein labeled insulin for binding to soluble human insulin receptor ectodomain. Figure 26A shows the binding curve. Figure 26B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

Figures 27A-27B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptides to compete with fluorescein labeled insulin for binding to soluble human insulin mini-receptor. Figure 27A shows the binding curve. Figure 27B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

Figure 28: A schematic drawing for the construction of protein fusions of the maltose binding protein.

Figure 29: BIAcore analysis of competition binding between IR and maltose binding protein fusion peptides H2C-9aa-H2C, H2C, and H2C-3aa-H2C.

Figure 30: Stimulation of IR autophosphorylation in vivo by maltose binding protein fusion peptides.

Figures 31A-31C: Results of free fat cell assays for insulin and Site 2-Site 1 peptides, S519 and S520. Figure 31A shows the results for S519. Figure 31B shows the results for S520. Figure 31C shows the EC_{50} values.

Figures 32A-32B: Results of human insulin receptor kinase assays for insulin and Site 2-Site 1 peptides S519 and S520. Figure 32A shows the substrate phosphorylation curve. Figure 32B shows the calculated Bestfit values.

Figure 33: Results of *in vivo* experiments showing the effect of intravenous administration of Site 2-Site 1 peptide S519 in Wistar rats:

Figures 34A-34E: Results of phage competition studies with IGF-1 peptides RP9 (Site 1) and D815 (Site 2). Phage: RP9 (A6-like); RP6 (B6-like); D8B12 (Site 2); and D815 (Site 2); Peptides: RP9 and D815. Figures 34A-34B show the competition curves. Figures 34C-34E show the symbol keys and peptide groups.

Figure 35A-35E: Phage competition studies with Site 2-Site 1 dimer peptides containing 6- or 12-amino acid linkers. Phage: RP9, RP6, D8B12, and D815; Peptides: D815-6L-RP9 and D815-12L-RP9. Figures 35A-35B show the competition curves. Figures 35C-35E show the symbol keys and peptide groups.

Figure 36: Results of IGF-1 agonist assay using FDC-P2 cells. Site 1 peptides RP6, RP9, G33, and Site 2 peptide D815 were tested in the agonist assay.

Figure 37: Results of IGF-1 antagonist assay using FDCP-2 cells. Site 1 peptides RP6, RP9, G33, and Site 2 peptide D815 were tested in the antagonist assay.

Figure 38: Results of IGF-1 agonist assay using FDCP-2 cells. Site 1 peptides 20E2, S175, and RP9 were tested in the agonist assay.

Figures 39: Results of agonist and antagonist studies with peptide monomers and dimers. Monomers: D815 and RP9; Dimers: D815-6aa-RP9 and D815-12aa-RP9.

Figure 40: Results of agonist and antagonist studies with peptide monomers and dimers. Monomers: G33 and D815; Dimer: D815-6aa-G33.

Figure 41: Results of agonist and antagonist studies with peptide monomers and dimers. Monomers: G33, D815 and RP9; Dimers: D815-6aa-RP9 and D815-12aa-RP9.

Figure 42: IGF-1 standard curve using FDC-P2 cells.

Figures 43A-43B: Peptide monomers identified from G33 and RP6 secondary libraries panned against IGF-1R (SEQ ID NOS:1262-1432).

Figure 43A shows peptides from G33 secondary library; Figure 43B shows peptides from RP6 secondary library.

Figures 44A-44B: Peptide dimers identified from libraries panned against IR or IGF-1R (SEQ ID NOS:1433-1540). Figure 44A shows dimer peptides panned against IR; Figure 44B shows dimer peptides panned against IGF-1R.

Figure 45: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide G33 (rG33) on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

Figure 46: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide D815 (rD815) on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

Figure 47: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide RP9 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

Figure 48: Results of heterogeneous time-resolved fluorometric assay showing the effect of recombinant peptide D815-6-G33 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

Figure 49: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide D815-6-RP9 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

Figure 50: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide D815-12-RP9 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

Figure 51: Results of heterogeneous time-resolved fluorometric assays showing the effect of IGF-1 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

Figure 52: Results of time-resolved fluorescence resonance energy transfer assays showing the effect of Site 1 peptides, Site 2 peptides, and rhIGF-1 on the dissociation of biotinylated-20E2 (b-20E2, Site 1) from recombinant human IGF-1R.

Figure 53: Results of time-resolved fluorescence resonance energy transfer assays showing the effect of various peptide monomers and dimers on the dissociation of biotinylated-20E2 (b-20E2, Site 1) from recombinant human IGF-1R.

Figures 54A-54B, 55A-55B, 56A-56B, 57A-57B, 58A-58B, 59A-59B, 60A-60C, 61A-61B, 62A-62B, 63A-63B, and 64A-64B: Amino acid sequences identified by panning peptide libraries against IGF-1R. The amino acids are represented by their one-letter abbreviation. The ratios over background are determined by dividing the signal at 405 nm (E-Tag, IGF-1R, or IR) by the signal at 405 nm for non-fat milk. The IGF-1R/IR ratio comparison is determined by dividing the ratio of IGF-1R by the ratio of IR. The IR/IGF-1R ratio comparison is determined by dividing the ratio of IR by the ratio of IGF-1R. Sp/Irr = the ratio of specific binding over non-specific binding; LDH = lactate dehydrogenase (negative control).

Where included, the design of each library is shown in the first line in bold. In the design, symbol 'X' indicates a random position, an underlined amino acid indicates a doped position at the nucleotide level, and other positions are held constant. Symbols in the listed sequences include: Q indicates a position corresponding to a TAG stop codon; # indicates a position corresponding to a TAA stop codon; * indicates a position corresponding to a TGA stop codon; and ? indicates an unknown amino acid. The Q residues represent translation read-through at TAG stop codons. All libraries were designed with the short FLAG® Epitope DYKD

(SEQ ID NO:1545; Hopp *et al.*, 1988, *Bio/Technology* **6**:1205-1210) at the N-terminus of the listed sequence and an E-tag epitope (GAPVPYPDPLEPR; SEQ ID NO:XX) at the C-terminus.

Figures 54A-54B: Peptides identified from a RP6 secondary library panned against IGF-1R. The RP9 peptide is a Formula 1, Site 1 monomer.

Figures 55A-55B: Peptides identified from a RP9-NPB25 secondary library panned against IGF-1R. The RP9-NPB25 peptide is a Formula 2, Site 1 monomer with a 25 amino acid C-terminal extension.

Figures 56A-56B: Peptides identified from a RP30-IGF-NPB20 secondary library panned against IGF-1R. The RP30-IGF-NPB20 peptide is a Site 1, Formula 2 monomer with a 20 amino acid C-terminal extension.

Figures 57A-57B: Peptides identified from a NPB20-RP30-IGF secondary library panned against IGF-1R. The NPB20-RP30-IGF peptide is a Site 1, Formula 2 monomer with a 20 amino acid N-terminal extension.

Figures 58A-58B: Peptides identified from a D815 secondary library panned against IGF-1R. The D815 peptide is a Formula 6, Site 2 monomer.

Figures 59A-59B: Peptides identified from a RP6-D815 secondary library panned against IGF-1R. The RP6-D815 peptide is a Site 1-Site 2 dimer with no linker.

Figures 60A-60C: Peptides identified from a RP6-6aa-D815 secondary library panned against IGF-1R. The RP6-6aa-D815 peptide is a Site 1-Site 2 dimer with a 6 amino acid linker.

Figures 61A-61B: Peptides identified from a RP6-RP9 secondary library panned against IGF-1R. The RP6-RP9 peptide is a Site 1-Site 1 dimer with no linker.

Figures 62A-62B: Peptides identified from a RP6-6aa-RP9 secondary library panned against IGF-1R. The RP6-6aa-RP9 peptide is a Site 1-Site 1 dimer with a 6 amino acid linker.

Figures 63A-63B: Peptides identified from a D815-RP6 secondary library panned against IGF-1R. The D815-RP6 peptide is a Site 2-Site 1 dimer with no linker.

Figures 64A-64B: Peptides identified from a D815-6aa-RP6 secondary library panned against IGF-1R. The D815-6aa-RP6 peptide is a Site 2-Site 1 dimer with a 6 amino acid linker.

Figures 65A-65F: Dose related increase in cell proliferation of MiaPaCa and MCF-7 cells as measured in response to IGF-1, IGF-2, and insulin. Cells were treated with either IGF-1, IGF-2, or insulin. Figure 65A: Results for MiaPaCa cells incubated with IGF-1; Figure 65B: MiaPaCa cells incubated with IGF-2; Figure 65C: MiaPaCa cells incubated with insulin; Figure 65D: MCF-7 cells incubated with IGF-1; Figure 65E: MCF-7 cells incubated with IGF-2; Figure 65F: MCF-7 cells incubated with insulin.

Figures 66A-66C: Peptide RP33-IGF competes with IGF-1 binding for binding to IGF-1R and antagonizes receptor activity in cell-based assays. For competition experiments, the ALPHAScreen assay format was used (see below). For antagonism assays, RP33-IGF was added to cells, cells were incubated with IGF-1, and cell number was determined. Figure 66A: Inhibition of IGF-1 binding as a function of RP33-IGF concentration. Figure 66B: Antagonism of IGF-1R in MCF-7 cells by peptide RP33-IGF. Figure 66C: Antagonism of IGF-1R in MiaPaCa cells by peptide RP33-IGF.

Figures 67A-67B: IGF-1 stimulates transient phosphorylation of IRS-1 in MCF-7 cells. Cells were stimulated with IGF-1 for 0, 2, 10, 30, 60 minutes and total protein was immunoprecipitated for each analysis. Figure 67A: Western blot analysis of endogenous IRS-1; Figure 67B: Western blot analysis of phosphorylated IRS-1; Lane 1: No addition; Lane 2: 2 minute time point; Lane 3: 10 minute time point; Lane 4: 30 minute time point; Lane 5: 60 minute time point.

Figures 68A-68B: Phosphorylation of IRS-1 in MCF-7 cells induced by IGF-1 is dose-dependant. Cells were exposed to increasing concentrations of IGF-1 and total protein was immunoprecipitated. Stimulation by 0.50 nM IGF-1 resulted in a sub-maximal level of phosphorylation that was consistently visualized in Western blot analysis. Figure 68A: Western blot analysis of endogenous IRS-1; Figure 68B:

Western blot analysis of phosphorylated IRS-1; Lane 1: No addition; Lane 2: 0.05 nM IGF-1; Lane 3: 0.1 nM IGF-1; Lane 4: 5 nM IGF-1; Lane 5: 1 nM IGF-1; Lane 6: 0.5 nM IGF-1; Lane 7: 10 nM IGF-1; Lane 8: 50 nM IGF-1.

Figures 69A-69B: Blockade of IGF-1-induced phosphorylation of IRS-1 in MCF-7 cells by synthetic peptides RP6KK and RP33-IGF. Unrelated peptides KCB1 (VSIGECGGLRHHRVRELCLV; SEQ ID NO:XX) and DGI3-D1 (ECRWFRPWRCPGLLSTGGGR; SEQ ID NO:XX) were used as negative controls. Figure 69A: Western blot analysis of expressed IRS-1; Figure 69B: Western blot analysis of phosphorylated IRS-1. Lane 1: no addition; Lane 2: DGI3-D1; Lane 3: KCB1; Lane 4: IGF-1 plus DGI3-D1; Lane 5: IGF-1 plus KCB1; Lane 6: IGF-1 plus RP6KK; Lane 7: IGF-1 plus RP33-IGF; Lane 8: IGF-1.

Figures 70A-70C: Peptides RP54 and RP52 compete with IGF-1 for binding to IGF-1R, and act as antagonists in cell proliferation assays. For antagonism assays, RP54 or RP52 was added to cells, cells were incubated with IGF-1, and cell number was determined. Figure 70A: Antagonism of IGF-1R by RP54 in MCF-7 cells; Figure 70B: Antagonism of RP54 in MiaPaCa cells. Figure 70C: Antagonism of IGF-1 by RP52 in MCF-7 cells.

Figures 71A-71F: Peptide monomers with IGF-1R agonist or antagonist activity in MCF-7 or MiaPaCa cell proliferation assays compete against IGF-1 for binding to IGF-1R. Potencies of peptide competition were determined using the AlphaScreen assay format (see below). Figure 71A: RP60 peptide; Figure 71B: RP48 peptide; Figure 71C: sG33 peptide; Figure 71D: C1 peptide; Figure 71E: L-RP9ex peptide; Figure 71F: 12-RP9ex peptide.

Figures 72A-72E: Peptide dimers with IGF-1R agonist activity in MCF-7 or MiaPaCa cell proliferation assays compete with IGF-1 for binding to IGF-1R. Potencies of peptide competition were determined using the AlphaScreen assay format (see below). Figure 72A: rRP30-IGF-12-D112 peptide (Site 1-Site 1); Figure 72B: rRP30-IGF-12-RP31-IGF peptide (Site

1-Site 2); Figure 72C: rRP31-IGF-12-RP30-IGF peptide (Site 2-Site 1); Figure 72D: rD112-12-RP30-IGF peptide (Site 1-Site 1); Figure 72E: rD112-12-D112 peptide (Site 1-Site 1).

Figures 73A-73D: Peptide monomers with IGF-1R agonist activity in MCF-7 or MiaPaCa cell proliferation assays. Figure 73A: RP60 peptide; Figure 73B: RP48 peptide; Figure 73C: G33 peptide; Figure 73D: L-RP9ex peptide.

Figures 74A-74I: Peptide dimers with IGF-1R agonist activity in MCF-7 or MiaPaCa cell proliferation assays. Figure 74A: RP30-IGF-12-D112 (Site 1-Site 1); Figure 74B: RP30-IGF-12-RP31-IGF (Site 1-Site 2); Figure 74C: RP31-IGF-12-RP30-IGF (Site 2-Site 1); Figure 74D: D112-12-RP30-IGF (Site 1-Site 1); Figure 74E: RP6-L-D8B12 (Site 1-Site 2); Figure 74F: D8B12-12-RP9 (Site 2-Site 1); Figure 74G: D112-12-D112 (Site 1-Site 1); Figure 74H: RP9-12-RP9 (Site 1-Site 1); Figure 74I: RP9-L-RP6 (Site 1-Site 1).

V. DETAILED DESCRIPTION OF THE INVENTION

This invention relates to amino acid sequences comprising motifs that bind to the insulin receptor (IR) and/or insulin-like growth factor receptor (IGF-1R). In addition to binding to IR and/or IGF-1R, the amino acid sequences also possess either agonist, partial agonist or antagonist activity at IR or IGF-1R. In addition, the amino acid sequences bind to separate binding sites (Sites 1 or 2) on IR or IGF-1R.

Although capable of binding to IR or IGF-1R at sites which participate in conferring agonist or antagonist activity, the amino acid sequences are not based on the native insulin or IGF-1 sequences, nor do they reflect an obvious homology to any such sequences.

The amino acid sequences of the invention may be peptides, polypeptides, or proteins. These terms as used herein should not be considered limiting with respect to the size of the various amino acid sequences referred to herein and which are encompassed within this

invention. Thus, any amino acid sequence comprising at least one of the IR or IGF-1R binding motifs disclosed herein, and which binds to IR or IGF-1R is within the scope of this invention. In preferred embodiments, the amino acid sequences confer insulin or IGF-1 agonist or antagonist activity. The amino acid sequences of the invention are typically artificial, i.e., non-naturally occurring peptides, polypeptides, or fragments thereof. The amino acid sequences of the invention do not include insulin, insulin-like growth factors, antibodies against insulin receptors or insulin-like growth factor receptors, or fragments thereof. Amino acid sequences useful in the invention may be obtained through various means such as chemical synthesis, phage display, cleavage of proteins or polypeptides into fragments, or by any means which amino acid sequences of sufficient length to possess binding ability may be made or obtained.

The amino acid sequences provided by this invention should have an affinity for IR sufficient to provide adequate binding for the intended purpose. Thus, for use as a therapeutic, the peptide, polypeptide, or protein provided by this invention should have an affinity (K_d) of between about 10^{-7} to about 10^{-15} M. More preferably the affinity is 10^{-8} to about 10^{-12} M. Most preferably, the affinity is 10^{-10} to about 10^{-12} M. For use as a reagent in a competitive binding assay to identify other ligands, the amino acid sequence preferably has affinity for the receptor of between about 10^{-5} to about 10^{-12} M.

The present invention describes several different binding motifs, which bind to active sites on IR or IGF-1R. The binding motifs are defined based on the analysis of several different amino acid sequences and analyzing the frequency that particular amino acids or types of amino acids occur at a particular position of the amino acid sequence as described in the related applications of Beasley *et al.* International Application PCT/US00/08528, filed March 29, 2000, and Beasley *et al.*, U.S. Application Serial No. 09/538,038, filed March 29, 2000.

Also included within the scope of this invention are amino acid sequences containing substitutions, additions, or deletions based on the teachings disclosed herein and which bind to IR or IGF-1R with the same or altered affinity. For example, sequence tags (e.g., FLAG® tags) or amino acids, such as one or more lysines, can be added to the peptide sequences of the invention (e.g., at the N-terminal or C-terminal ends) as described in detail herein. Sequence tags can be used for peptide purification or localization. Lysines can be used to increase peptide solubility or to allow for biotinylation. Alternatively, amino acid residues located at the carboxy and amino terminal regions of the consensus motifs described below, which comprise sequence tags (e.g., FLAG® tags), or which contain amino acid residues that are not associated with a strong preference for a particular amino acid, may optionally be deleted providing for truncated sequences. Certain amino acids (e.g., C-terminal or N-terminal residues) such as lysine which promote the stability or biotinylation of the amino acids sequences may be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble, or linked to a solid support.

Peptides that bind to IR or IGF-1R, and methods and kits for identifying such peptides, have been disclosed by Beasley *et al.*, International Application PCT/US00/08528 filed March 29, 2000 and Beasley *et al.*, U.S. Application Serial No. 09/538,038 filed March 29, 2000, which are incorporated by reference in their entirety.

A. Consensus Motifs

The following motifs have been identified as conferring binding activity to IR and/or IGF-1R:

1. $X_1X_2X_3X_4X_5$ (Formula 1; Group 1; the A6 motif) wherein X_1, X_2, X_4 and X_5 are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, X_1 and X_5 are phenylalanine and X_2 is tyrosine. X_3 may be

any small polar amino acid, but is preferably selected from aspartic acid, glutamic acid, glycine, or serine, and is most preferably aspartic acid or glutamic acid. X_4 is most preferably tryptophan, tyrosine, or phenylalanine and most preferably tryptophan. Particularly preferred embodiments of the A6 motif are FYDWF (SEQ ID NO:1554) and FYEWF (SEQ ID NO:1555). The A6 motif possesses agonist activity at IGF-1R, but agonist or antagonist activity at IR depending on the identity of amino acids flanking A6. See Figure 5A.

Amino acid sequences that comprise the A6 motif and possess agonist activity at IR, include but are not limited to, D117/H2C: FHENFYDWFVRQVSKK (SEQ ID NO:1556); D117/H2 minus terminal lysines: FHENFYDWFVRQVS (SEQ ID NO:1557); RP9: GSLDESFYDWFERQLGKK (SEQ ID NO:1558); RP9 minus terminal lysines: GSLDESFYDWFERQLG (SEQ ID NO:1559); and S175: GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560). Preferred RP9 sequences include GLADEDFYEWFERQLR (SEQ ID NO:1561), GLADELFYEWFDRQLS (SEQ ID NO:1562), GQLDEDFYEWFDRQLS (SEQ ID NO:1563), GQLDEDFYAWFDRQLS (SEQ ID NO:1564), GFMDESFYEWFERQLR (SEQ ID NO:1565), GFWDESFYAWFERQLR (SEQ ID NO:1566), GFMDESFYAWFERQLR (SEQ ID NO:1567), and GFWDESFYEWFERQLR (SEQ ID NO:1568). Non-limiting examples of Group 1 (Formula 1; A6) amino acid sequences are shown in Figures 1A-1O.

2. $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ (Formula 2, Group 3; the B6 motif) wherein X_6 and X_7 are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, X_6 is phenylalanine and X_7 is tyrosine. X_8, X_9, X_{11}, and X_{12} may be any amino acid. X_{10} and X_{13} are hydrophobic amino acids, preferably leucine, isoleucine, phenylalanine, tryptophan or methionine, but more preferably leucine or isoleucine. X_{10} is most preferably isoleucine for binding to IR and leucine for binding to IGF-1R. X_{13} is most preferably leucine. Amino acid sequences of Formula 2 may function as an antagonist

at the IGF-1R, or as an agonist at the IR. Preferred consensus sequences of the Formula 2 motif are $FYX_8X_9LX_{11}X_{12}L$ (SEQ ID NO:1569), $FYX_8X_9IX_{11}X_{12}L$ (SEQ ID NO:1570), $FYX_8AIX_{11}X_{12}L$ (SEQ ID NO:1571), and $FYX_8YFX_{11}X_{12}L$ (SEQ ID NO:1572).

Another Formula 2 motif for use with this invention comprises $FYX_8Y FX_{11}X_{12}L$ (SEQ ID NO:1573) and is shown as Formula 2A ("NNRP") below: $X_{115}X_{116}X_{117}X_{118}FYX_8YFX_{11}X_{12}LX_{119}X_{120}X_{121}X_{122}$ (SEQ ID NO:1574) wherein X_{115}-X_{118} and X_{118}-X_{122} may be any amino acid which allows for binding to IR or IGF-1R. X_{115} is preferably selected from the group consisting of tryptophan, glycine, aspartic acid, glutamic acid, and arginine. Aspartic acid, glutamic acid, glycine, and arginine are more preferred. Tryptophan is most preferred. The preference for tryptophan is based on its presence in clones at a frequency three to five fold higher than that expected over chance for a random substitution, whereas aspartic acid, glutamic acid and arginine are present about two fold over the frequency expected for random substitution.

X_{116} preferably is an amino acid selected from the group consisting of aspartic acid, histidine, glycine, and asparagine. X_{117} and X_{118} are preferably glycine, aspartic acid, glutamic acid, asparagine, or alanine. More preferably X_{117} is glycine, aspartic acid, glutamic acid and asparagine whereas X_{118} is more preferably glycine, aspartic acid, glutamic acid or alanine. X_8 when present in the Formula 2A motif is preferably arginine, glycine, glutamic acid, or serine. X_{11} when present in the Formula 2A motif is preferably glutamic acid, asparagine, glutamine, or tryptophan, but most preferably glutamic acid. X_{12} when present in the Formula 2A motif is preferably aspartic acid, glutamic acid, glycine, lysine or glutamine, but most preferably aspartic acid. X_{119} is preferably glutamic acid, glycine, glutamine, aspartic acid or alanine, but most preferably glutamic acid. X_{120} is preferably glutamic acid, aspartic acid, glycine or glutamine, but most preferably glutamic acid. X_{121} is preferably tryptophan, tyrosine, glutamic acid, phenylalanine, histidine, or aspartic acid, but most preferably tryptophan or tyrosine. X_{122} is preferably glutamic acid, aspartic acid or glycine; but most

preferably glutamic acid. Preferred amino acid residue are identified based on their frequency in clones over two fold over that expected for a random event, whereas the more preferred sequences occur about 3-5 times as frequently as expected.

In certain cases, Formula 1 and Formula 2 amino acid sequences may also include two cysteine residues, which may be positioned either outside or inside the motif sequence (e.g., X_1 X_2 X_3 X_4 X_5 and $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$), as described herein. The spacing between the cysteine residues preferably may vary from 3 amino acids, e.g., RP62 (CDFYCALSRLSGQPRDRMPNYPGTS; SEQ ID NO:XX) up to 19 amino acids, e.g., RP35 (DRDFCRFYERLTALVGGQVDGGWPC; SEQ ID NO:XX). Formula 1 and Formula 2 peptides may exhibit varying size and cysteine positioning. For example, Formula 2 peptide RP6 (TFYSCLASLLTGTPQPNRGPWERCR; SEQ ID NO:XX) and derivatives, RP30-IGF, RP33-IGF, include two cysteine residues separated by 18 amino acids. In contrast, Formula 1 peptide G33 (GIISQSCPESFYDWFAGQVSDPWWCW; SEQ ID NO:XX) includes two cysteines separated by 17 amino acid residues. In certain Formula and Formula 2 peptides, the position and spacing of the cysteine residues was found to be highly preferred in these peptides as determined by calculations of amino acid preferences from peptides obtained by biopanning of RP6 and G33 secondary libraries. Without wishing to be bound by theory, it is possible that the cysteine pairs observed in Formula 1 and Formula 2 amino acid sequences form cysteine loop structures.

3. $X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}$ (Formula 3, reverse B6, revB6), wherein X_{14} and X_{17} are hydrophobic amino acids; X_{14}, X_{17} are preferably leucine, isoleucine, and valine, but most preferably leucine; X_{15}, X_{16}, X_{18} and X_{19} may be any amino acid; X_{20} is an aromatic amino acid, preferably tyrosine or histidine, but most preferably tyrosine; and X_{21} is an aromatic amino acid, but preferably phenylalanine or tyrosine, and most preferably

phenylalanine. For use as an IGF-1R binding ligand, an aromatic amino acid is strongly preferred at X_{18}.

4. $X_{22}X_{23}X_{24}X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}$ (Formula 4; Group 7; the F8 motif) wherein X_{22}, X_{25}, X_{26}, X_{28}, X_{29}, X_{30}, X_{33}, X_{34}, X_{35}, X_{36}, X_{37}, X_{38}, X_{40}, and X_{41} are any amino acid. X_{35} and X_{37} may be any amino acid when the F8 motif is used as an IR binding ligand or as a component of an IR binding ligand, however for use as an IGF-1R binding ligand, glycine is strongly preferred at X_{37} and a hydrophobic amino acid, particularly, leucine, is preferred at X_{35}. X_{23} is a hydrophobic amino acid. Methionine, valine, leucine or isoleucine are preferred amino acids for X_{23}, however, leucine which is most preferred for preparation of an IGF-1R binding ligand is especially preferred for preparation of an IR binding ligand. At least one cysteine is located at X_{24} through X_{27}, and one at X_{39} or X_{40}. Together the cysteines are capable of forming a cysteine cross-link to create a looped amino acid sequence. In addition, although a spacing of 14 amino acids in between the two cysteine residues is preferred, other spacings may also be used provided binding to IGF-1R or IR is maintained. Accordingly, other amino acids may be substituted for the cysteines at positions X_{24} and X_{39} if the cysteines occupy other positions.

In one embodiment, for example, the cysteine at position X_{24} may occur at position X_{27} which will produce a smaller loop provided that the cysteine is maintained at position X_{39}. These smaller looped peptides are described herein as Formula 5, infra. X_{27} is any polar amino acid, but is preferably selected from glutamic acid, glutamine, aspartic acid, asparagine, or as discussed above cysteine. The presence of glutamic acid at position X_{27} decreases binding to IR but has less of an effect on binding to IGF-1R. X_{31} is any aromatic amino acid and X_{32} is any small amino acid. For binding to IGF-1R, glycine or serine is preferred at position X_{31}, however, tryptophan is highly preferred for binding to IR. At position X_{32}, glycine is preferred for both IGF-1R and IR binding. X_{36} is an aromatic amino acid. A preferred consensus sequence for F8 is $X_{22}LCX_{25}X_{26}EX_{28}X_{29}X_{30}WGX_{33}X_{34}X_{35}$

$X_{36}X_{37}X_{38}CX_{40}X_{41}$ (SEQ ID NO:1575) whereas the amino acids are defined above. A more preferred F8 sequence is HLCVLEELFWGASLFGYCSG ("F8"; SEQ ID NO:1576). Amino acid sequences comprising the F8 sequence motif preferably bind to IR over IGF-1R. Figures 2A-2E list non-limiting examples of Formula 4 amino acid sequences.

5.

$X_{42}X_{43}X_{44}X_{45}X_{46}X_{47}X_{48}X_{49}X_{50}X_{51}X_{52}X_{53}X_{54}X_{55}X_{56}X_{57}X_{58}X_{59}X_{60}X_{61}$ (Formula 5; mini F8 motif) wherein X_{42}, X_{43}, X_{44}, X_{45}, X_{53}, X_{55}, X_{56}, X_{58}, X_{60}, and X_{61} are any amino acid. X_{43}, X_{46}, X_{49}, X_{50}, and X_{54} are hydrophobic amino acids, however, X_{43} and X_{46} are preferably leucine, whereas X_{50} is preferably phenylalanine or tyrosine but most preferably phenylalanine. X_{47} and X_{59} are cysteines. X_{48} is preferably a polar amino acid, i.e., aspartic acid or glutamic acid, but most preferably glutamic acid. Use of the small amino acid at position 54 may confer IGF-1R specificity. X_{51}, X_{52}, and X_{57} are small amino acids, preferably glycine. A preferred consensus sequence for mini F8 is $X_{42}X_{43}X_{44}X_{45}$LCEX_{49}FGG$X_{53}X_{54}X_{55}X_{56}$G $X_{58}CX_{60}X_{61}$ (SEQ ID NO:1577). Amino acid sequences comprising the sequence of Formula 5 preferably bind to IGF-1R or IR.

6. $X_{62}X_{63}X_{64}X_{65}X_{66}X_{67}X_{68}X_{69}X_{70}X_{71}X_{72}X_{73}X_{74}X_{75}X_{76}X_{77}X_{78}X_{79}X_{80}$ X_{81} (Formula 6; Group 2; the D8 motif) wherein X_{62}, X_{65}, X_{68}, X_{69}, X_{71}, X_{73}, X_{76}, X_{77}, X_{78}, X_{80}, and X_{81} may be any amino acid. X_{66} may also be any amino acid, however, there is a strong preference for glutamic acid. Substitution of X_{66} with glutamine or valine may result in attenuation of binding. X_{63}, X_{70}, and X_{74} are hydrophobic amino acids. X_{63} is preferably leucine, isoleucine, methionine, or valine, but most preferably leucine. X_{70} and X_{74} are preferably valine, isoleucine, leucine, or methionine. X_{74} is most preferably valine. X_{64} is a polar amino acid, more preferably aspartic acid or glutamic acid, and most preferably glutamic acid. X_{67} and X_{75} are aromatic amino acids. Whereas tryptophan is highly preferred at X_{67}, X_{75} is preferably tyrosine or tryptophan but most preferably tyrosine. X_{72} and X_{79} are

cysteines that again are believed to form a loop which position amino acid may be altered by shifting the cysteines in the amino acid sequence.

D8 is most useful as an amino acid sequence having a preference for binding to IR as only a few D8 sequences capable of binding to IGF-1R over background have been detected. A preferred sequence for binding to IR is $X_{62}LX_{64}X_{65}X_{66}WX_{68}X_{69}X_{70}X_{71}CX_{73}X_{74}X_{75}X_{76}X_{77}X_{78}CX_{80}X_{81}$ (SEQ ID NO:1578). Examples of specific peptide sequences comprising this motif include D8: KWLDQEWAWVQCEVYGRGCPSKK (SEQ ID NO:1579); and D8 minus terminal lysines: KWLDQEWAWVQCEVYGRGCPS (SEQ ID NO:1580). Preferred D8 monomer sequences include SLEEEWAQIQCEIYGRGCRY (SEQ ID NO:1581) and SLEEEWAQIQCEIWGRGCRY (SEQ ID NO:1582). Preferred D8 dimer sequences include SLEEEWAQIECEVYGRGCPS (SEQ ID NO:1583), and SLEEEWAQIECEVWGRGCPS (SEQ ID NO:1584). Non-limiting examples of Group 2 (Formula 6; D8) amino acid sequences are shown in Figures 3A-3E.

7. $HX_{82}X_{83}X_{84}X_{85}X_{86}X_{87}X_{88}X_{89}X_{90}X_{91}X_{92}$ (Formula 7) wherein X_{82} is proline or alanine but most preferably proline; X_{83} is a small amino acid more preferably proline, serine or threonine and most preferably proline; X_{84} is selected from leucine, serine or threonine but most preferably leucine; X_{85} is a polar amino acid preferably glutamic acid, serine, lysine or asparagine but more preferably serine; X_{86} may be any amino acid but is preferably a polar amino acid such as histidine, glutamic acid, aspartic acid, or glutamine; X_{87} is an aliphatic amino acid preferably leucine, methionine or isoleucine and most preferably leucine; amino acid X_{88}, X_{89} and X_{90} may be any amino acids; X_{91} is an aliphatic amino acid with a strong preference for leucine as is X_{92}. Phenylalanine may also be used at position 92. A preferred consensus sequence of Formula 7 is $HPPLSX_{86}LX_{88}X_{89}X_{90}LL$ (SEQ ID NO:1585). The Formula 7 motif binds to IR with little or no binding to IGF-1R.

8. Another sequence is $X_{104}X_{105}X_{106}X_{107}X_{108}X_{109}X_{110}X_{111}X_{112}X_{113}X_{114}$ (Formula 8) which comprises eleven amino acids wherein at least one, and preferably two of the amino acids of X_{106} through X_{111} are tryptophan. In addition, it is also preferred that when two tryptophan amino acids are present in the sequence they are separated by three amino acids, which are preferably, in sequential order proline, threonine and tyrosine with proline being adjacent to the tryptophan at the amino terminal end. Accordingly, the most preferred sequence for $X_{107}X_{108}X_{109}X_{110}X_{111}$ is WPTYW (SEQ ID NO:1586). At least one of the three amino acids on the amino terminal (X_{104}, X_{105}, X_{106}) and at least one of the amino acids carboxy terminal (X_{112}, X_{113}, X_{114}) ends immediately flanking X_{107}-X_{111} are preferably a cysteine residue, most preferably at X_{105} and X_{113} respectively. Without being bound by theory, the cysteines are preferably spaced so as to allow for the formation of a loop structure. X_{104} and X_{114} are both small amino acids such as, for example, alanine and glycine. Most preferably, X_{104} is alanine and X_{114} is glycine. X_{105} may be any amino acid but is preferably valine. X_{112} is preferably asparagine. Thus, the most preferred sequence is ACVWPTYWNCG (SEQ ID NO:1587).

9. An amino acid sequence comprising JBA5: DYKDLCQSWGVRIGWLAGLCPKK (SEQ ID NO:1541); or JBA5 without terminal lysines: LCQSWGVRIGWLAGLCP (SEQ ID NO:1542) (Formula 9). The Formula 9 motif is another motif believed to form a cysteine loop that possesses agonist activity at both IR and IGF-1R. Although IR binding is not detectable by ELISA, binding of Formula 9 to IR is competed by insulin and is agonistic.

10. W X_{123} G Y X_{124} W X_{125} X_{126} (SEQ ID NO:1543) (Formula 10; Group 6) wherein X_{123} is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; X_{124} is any amino acid, but preferably a charged or aromatic amino acid; X_{125} is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. X_{126} is any amino acid, but preferably a small amino acid. In one embodiment of

the present invention, the Formula 10, Group 6 motif is WPGY (SEQ ID NO:1588). Examples of specific peptide sequences comprising this motif include E8: KVRGFQGGTVWPGYEWLRNAAKK (SEQ ID NO:1589); and E8 minus terminal lysines: KVRGFQGGTVWPGYEWLRNAA (SEQ ID NO:1590). Preferred Group 6 sequences include WAGYEWF (SEQ ID NO:1591), WEGYEWL (SEQ ID NO:1592), WAGYEWL (SEQ ID NO:1593), WEGYEWF (SEQ ID NO:1594), and DSDWAGYEWFEEQLD (SEQ ID NO:1595). Non-limiting examples of Group 6 amino acid sequences are shown in Figures 4A-4B.

The IR and IGF-1R binding activities of representative Group 1 (Formula 1; A6); Group 2 (Formula 6; D8); and Group 6 (Formula 10); and Group 7 (Formula 4; F8) amino acid sequences are summarized in Figures 8 and 9A-9B. Group 1 (Formula 1; A6) amino acid sequences contain the consensus sequence FyxWF (SEQ ID NO:1596), which is typically agonistic in cell-based assays. Group 2 (Formula 6; D8) amino acid sequences are composed of two internal sequences having a consensus sequence VYGR (SEQ ID NO:1597) and two cysteine residues each. Thus, Group 2 peptides are capable of forming a cyclic peptide bridged with a disulfide bond. Neither of these consensus sequences have any significant linear sequence similarities greater than 2 or 3 amino acids with mature insulin. Group 7 (Formula 4; F8) amino acid sequences are composed of two internal exemplary sequences which do not have any significant sequence homology, but have two cysteine residues 13-14 residues apart, thus being capable of forming a cyclic peptide with a long loop anchored by a disulfide bridge.

B. Amino And Carboxyl Terminal Extensions Modulate Activity of Motifs

In addition to the motifs stated above, the invention also provides preferred sequences at the amino terminal or carboxyl terminal ends which are capable of enhancing binding of the motifs to either IR, IGF-1R, or both.

In addition, the use of the extensions described below does not preclude the possible use of the motifs with other substitutions, additions or deletions that allow for binding to IR, IGF-1R, or both.

1. Formula 1

Any amino acid sequence may be used for extensions of the amino terminal end of A6, although certain amino acids in amino terminal extensions may be identified which modulate activity. Preferred carboxy terminal extensions for A6 are A6-$X_{93}X_{94}X_{95}X_{96}X_{97}$ wherein X_{93} may be any amino acid, but is preferably selected from the group consisting of alanine, valine, aspartic acid, glutamic acid, and arginine, and X_{94} and X_{97} are any amino acid; X_{95} is preferably glutamine, glutamic acid, alanine or lysine but most preferably glutamine. The presence of glutamic acid at X_{95} however may confer some IR selectivity. Further, the failure to obtain sequences having an asparagine or aspartic acid at position X_{95} may indicate that these amino acids should be avoided to maintain or enhance sufficient binding to IR and IGF-1R. X_{96} is preferably a hydrophobic or aliphatic amino acid, more preferably leucine, isoleucine, valine, or tryptophan but most preferably leucine. Hydrophobic residues, especially tryptophan at X_{96} may be used to enhance IR selectivity.

2. Formula 2

B6 with amino terminal and carboxy terminal extensions may be represented as $X_{98}X_{99}$-B6-X_{100}. X_{98} is optionally aspartic acid and X_{99} is independently an amino acid selected from the group consisting of glycine, glutamine, and proline. The presence of an aspartic acid at X_{98} and a proline at X_{99} is associated with an enhancement of binding for both IR and IGF-1R. A hydrophobic amino acid is preferred for the amino acid at X_{100}, an aliphatic amino acid is more preferred. Most preferably leucine, for IR and valine for IGF-1R. Negatively charged amino acids are preferred at both the amino and carboxy terminals of Formula 2A.

3. Formula 3

An amino terminal extension of Formula 3 defined as $X_{101}X_{102}X_{103}$-revB6 wherein X_{103} is a hydrophobic amino acid, preferably leucine, isoleucine or valine, and X_{102} and X_{101} are preferably polar amino acids, more preferably aspartic acid or glutamic acid may be useful for enhancing binding to IR and IGF-1R. No preference is apparent for the amino acids at the carboxy terminal end of Formula 3.

4. Formula 10

In one preferred embodiment, Formula 10 sequences $WX_{123}GYX_{124}WX_{125}X_{126}$ (SEQ ID NO:1543) can include an amino terminal extension comprising the sequence DSD and/or a carboxy terminal extension comprising the sequence EQLD (SEQ ID NO:1598).

C. IR Binding Preferences

As indicated above, the amino acid sequences containing the motifs of this invention may be constructed to have enhanced selectivity for either IR or IGF-1R by choosing appropriate amino acids at specific positions of the motifs or the regions flanking them. By providing amino acid preferences for IR or IGF-1R, this invention provides the means for constructing amino acid sequences with minimized activity at the non-cognate receptor. For example, the amino acid sequences disclosed herein with high affinity and activity for IR and low affinity and activity for IGF-1R are desirable as IR agonist as their propensity to promote undesirable cell proliferation, an activity of IGF-1 agonists, is reduced. Ratios of IR binding affinity to IGF-1R binding affinity for specific sequences are provided in Figures 1A-1O; 2A-2E; 3A-3E; 4A-4I; 44A-44B. As an insulin therapeutic, the IR/IGF-1R binding affinity ratio is preferably greater than 100. Conversely, for use as an IGF-1R therapeutic, the IR/IGF-1R ratio should be less than 0.01. Examples of peptides that selectively bind to IGF-1R are shown below.

TABLE 1

IGF-1R-SELECTIVE SEQUENCES **FORMULA 1 (Group 1; A6-like):**

			Ratios over Background			Comparisons	
Clone	**SEQ ID NO:**	**Sequence**	**E-Tag**	**IGF-1R**	**IR**	**IGF-1R/IR**	**IR/IGF-1R**
A6L-0-E6-IR	1599	YRGMLVLGRSSDGAGKVAFERPARIGQTVFAVNFYDWFV	31.0	31.0	1.8	17.0	0.1
H2CA-4-G9-IGFR	1600	GIISQSCPESFYDWFAGQVSDPWWCW	8.6	9.5	0.6	16.0	0.1
H2CA-4-H6-IGFR	1601	VGRASGFPENFYDWFGRQLSLQSGEQ	4.9	10.5	0.7	14.6	0.1
A6L-0-E4-IR	1602	YRGMLVLGRISDGAG#VASEPPARIGRKVFAVNFYDWFV	26.0	16.0	1.3	13.0	0.1
A6L-0-H3-IR	1603	YRGMLVLGRISGGAGKAASERPARIGQKVSAVNFYDWFV	27.0	26.0	2.0	13.0	0.1
H2CA-4-F5-IGFR	1604	VGYQGQGDENFYDWFIRQVSGRLGVQ	5.5	9.7	0.8	12.3	0.1
H2CA-4-H8-IGFR	1605	SACQFDCHENFYDWFARQVSGGAAYG	5.6	9.2	1.0	9.4	0.1
H2CA-4-F11-IGFR	1606	SAAQLFFQESFYDWFLRQVAESSQPN	3.5	6.8	1.0	6.7	0.1
H2CA-4-F6-IGFR	1607	AVRATRFDEAFYDWFVRQISDGQGNK	3.9	7.3	1.1	6.4	0.2
H2CA-4-F10-IGFR	1608	VNQSGSIHENFYDWFERQVSHQRGVR	4.9	5.7	1.0	5.9	0.2
H2CA-1-A3-IGFR	1609	APDPSDFQEIFYDWFVRQVSRMPGGG	7.7	3.8	0.8	5.1	0.2
H2CA-3-C8-IGFR	1610	SSCDGAGHESFYEWFVRQVSGCRSV	15.1	5.6	1.2	4.8	0.2
H2CA-2-B9-IGFR	1611	RAGSSDFHEDFYEWFVRQVSLSLKGK	9.3	7.0	1.7	4.2	0.2
H2CA-4-H4-IGFR	1612	QAVQPGFHEEFYDWFVRQVSTGVGGG	3.9	4.1	1.0	4.2	0.2
E4Dα-4-H2-IR	1613	GFREGNFYEWFQAQVT	37.8	33.9	8.2	4.1	0.2
H2CA-4-F7-IGFR	1614	SSIGGGFHENFYDWFSRQLSQSPPLK	1.5	3.2	0.8	4.1	0.2
H2CA-3-D6-IGFR	1615	QSPVGSSHEDFYDWFFRQVAQSGAHQ	8.3	9.0	2.2	4.0	0.3
H2CA-3-D8-IGFR	1616	NYRRQVFNGNFYDWFDRQVFSLVTPG	10.9	7.2	1.8	4.0	0.3
H2CA-4-G11-IGFR	1617	TLDGGSFEEQFYDWFVRQLSYRTNPD	10.8	9.5	2.5	3.9	0.3
H2CA-4-F1-IGFR	1618	FYVQQWGHENFYDWFDRQVSQSGGAG	5.8	3.5	0.9	3.8	0.3
H2CA-3-D7-IGFR	1619	LRRQAPVEENFYDWFVRQVSGDRVGG	13.3	3.0	0.8	3.7	0.3
H2CA-1-A7-IGFR	1620	RCGRELYHSTFYDWFDRQVAGRTCPS	8.0	2.2	0.6	3.7	0.3
H2CA-2-B4-IGFR	1621	CCLLCRFQQNFYDWFVCQGISRLRPL	3.5	4.1	1.1	3.6	0.3
H2CA-2-B3-IGFR	1622	PPLASDLDVQFYGWFVQQVSPPGRGG	7.7	3.8	1.0	3.6	0.3
H2CA-2-B2-IGFR	1623	GAPVDQLHEDFYDWFVRQVSQAATG	4.1	3.4	1.0	3.5	0.3
E4Dα-2-D11-IR	1624	GFREGSFYDWFQAQVT	40.2	11.1	3.3	3.4	0.3
20E2Bβ-4-G6-IR	1625	SQAGSAFYAWFDQVLRTVHSA	22.4	6.2	1.9	3.3	0.3
H2CA-4-H9-IGFR	1626	RGAVAGFHDQFYDWFDRQVSRVHKFG	8.7	5.6	1.9	3.0	0.3

			Ratios over Background			Comparisons	
Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
H2CA-2-B11-IGFR	1627	AICDAGFHEHFYDWFALQVSDCGRQS	11.9	4.6	1.6	3.0	0.3
H2CA-3-E8-IGFR	1628	LGYQEPFQQNFYDWFVRQVSGAENAG	13.2	6.3	2.2	2.9	0.3
A6S-2-D11-IR	1629	EAASLGSQDRNFYDWFVRQVV	48.4	37.4	13.5	2.8	0.4
A6S-2-D1-IR	1630	VERSASSQDGNFYDWFVVQIR	37.8	30.6	12.0	2.6	0.4
A6S-3-E2-IR	1631	TSEVQRRSQDNFYDWFVAQVA	33.1	24.7	9.8	2.5	0.4
H2CA-3-E11-IGFR	1632	HLADGQFHEKFYDWFERQISSRCNDC	4.7	2.2	1.0	2.2	0.5
H2CA-3-C11-IGFR	1633	FRTLAAQHDSFYDWFDRQVSGAAGER	9.3	3.3	1.6	2.1	0.5
A6-PD1-IGFR	1634	SFHEDFYDWFDRQVSGSLKK					
H2C-PD1-IGFR(RP9)	1558	GSLDESFYDWFERQLGKK					

FORMULA 2 (Group 2; B6-like):

			Ratios over Background			Comparisons	
Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
20C-3-G3-IGFR	1635	TFYSCLASLLTGTPQPNRGPWERCR	33.1	32.3	1.2	27.0	<0.1
20C-4-C7-IGFR	1636	FFYDCLAALLQGVARYHDLCAVEIT	35.3	28.0	1.3	21.8	<0.1
B6Hα-1-B5-IR	1637	CCTTEMVVMDARDDPFYHKLSELVTGG	41.5	20.5	1.0	20.5	0.0
R20β-4-A6-IR	1638	RGQSDAFYSGLWALIGLSDG	9.3	25.9	1.5	17.3	0.1
20E2B-1-A6-IGFR	1639	GVRAMSFYDALVSVLGLGPSG	18.6	18.1	1.1	16.8	0.1
R20α-4-20A12-IR	1640	RLFYCGIQALGANLGYSGCV	48.6	39.9	2.4	16.6	0.1
20E2Bβ-4-G7-IR	1641	LQPCSGFYECIERLIGVKLSG	19.9	25.2	1.6	15.8	0.1
NNRPγ-4-B11-IR	1642	LKDGFYDYFWQRLHLGS	4.1	18.7	1.2	15.5	0.1
20E2B-3-C6-IGFR	1643	VEGRGLFYDLLRQLLARRQNG	17.9	16.8	1.1	14.8	0.1
B6Hα-1-A2-IR	1644	RGCNDDGGKGWSDDPFYHKLSELICGG	22.3	14.6	1.0	14.6	0.1
20E2A-4-F11-IGFR	1645	QGGSASFYDAIDRLLRMRIGG	21.3	18.8	1.3	14.6	0.1
B6Hα-3-E9-IR	1646	RCEEKQAEVGPSSDPFYHKMSELLGCR	44.6	24.2	1.7	14.2	0.1
20C-3-F6-IGFR	1647	DRDFCRFYERLTALVGGQVDGGWPC	33.5	26.1	1.9	14.1	0.1
20E2B-4-H3-IGFR	1648	KLHNLMFYYGLQRLVWGAGLG	11.2	14.8	1.1	13.9	0.1
20E2B-3-C2-IGFR	1649	GNGDGMFYQLLSLLVGRDMHV	13.1	8.9	0.6	13.8	0.1
20C-3-A1-IGFR	1650	SSYGCDGFYLMLFSLGLVASQELEC	26.5	20.8	1.5	13.7	0.1
20E2B-3-E3-IGFR	1651	PDLHKGFYAQLAQLIRGQLLS	22.4	16.3	1.3	13.1	0.1
R20α-3-20E2-IR	1652	FYDAIDQLVRGSARAGGTRD	46.3	39.9	3.1	12.9	0.1
20E2B-4-H12-IGFR	1653	YSCGDGFYSLLSDLLGGQFRC	6.5	9.7	0.8	12.8	0.1
B6Hα-3-F11-IR	1654	RGMKEEVLVGGSTDPFYHKLSELLQGS	49.5	18.7	1.6	11.7	0.1

			Ratios over Background			Comparisons	
Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
20E2B-3-D2-IGFR	1655	IQQELTFYDLLHRLVRSELGS	20.7	12.4	1.1	11.7	0.1
20E2B-3-D8-IGFR	1656	GGTEVDFYRALERLVRGQLGL	20.4	17.7	1.6	11.3	0.1
20E2B-3-E8-IGFR	1657	LRIANLFYQRLWDLAFGGGG	15.7	16.7	1.5	11.1	0.1
B6Hα-2-C4-IR	1658	RCGRW*AEMGAGDDPFYHKLSELVCG	20.7	9.9	0.9	11.0	0.1
R20α-4-20C11-IR	1659	DRAFYNGLRDLVGAVYGAWD	43.7	30.8	3.0	10.3	0.1
20E2B-4-F8-IGFR	1660	PVGVQGFYEGLSRLVLGRGGW	12.3	7.3	0.8	9.7	0.1
20E2B-1-A11-IGFR	1661	RFSTDGFYQYLLALVGGGPVG	15.0	9.5	1.0	9.7	0.1
20E2B-3-D4-IGFR	1662	NSRDGGFYLQLERLLGFPVTG	8.1	7.9	0.8	9.6	0.1
20E2B-2-B11-IGFR	1663	VVTPVNFYRALEALVRG.RLG	13.9	10.6	1.1	9.4	0.1
20E2B-3-C8-IGFR	1664	QPAPDGFYSALMKLIGRGGVS	18.5	15.6	1.8	8.9	0.1
20E2B-2-B2-IGFR	1665	PGTDLGFYQALRCVVIQGACD	11.7	4.9	0.6	8.1	0.1
20E2B-4-F10-IGFR	1666	AQPCGGFYGLLEQLVGRSVCD	19.0	17.3	2.2	7.8	0.1
20E2B-4-F9-IGFR	1667	QPDHSYFYSLLQELVGSEERL	11.9	14.7	1.9	7.7	0.1
20C-3-A4-IGFR	1668	QFYGCLLDLSLGVPSFGWRRRCITA	17.7	8.8	1.2	7.6	0.1
20E2B-3-D11-IGFR	1669	LGVTDGFYAALGYLIHGVGQF	14.3	12.2	1.6	7.6	0.1
20E2B-3-C11-IGFR	1670	CMM.DGFYAGLGCLLTAGEGR	15.3	15.4	2.1	7.5	0.1
20E2B-2-B3-IGFR	1671	ICTGQGFYQVLCGLLRGTSAR	9.1	5.3	0.7	7.4	0.1
20E2B-3-D12-IGFR	1672	QGNVLDFYGWIGRLLAKQGSD	10.3	6.2	0.9	7.3	0.1
20E2B-3-E12-IGFR	1673	VATSQGFYSGLSELLQGGGNV	13.9	6.0	0.8	7.3	0.1
20E2B-2-B8-IGFR	1674	IWATGDFYRLLSQLVMGRVGT	17.4	5.7	0.8	7.2	0.1
NNRPγ-4-A9-IR	1675	EGSGFYGYFFSLLGLQG	3.0	10.0	1.4	7.1	0.1
20E2B-4-G11-IGFR	1676	RQGTGSFYLMLEQLLVGARGP	8.9	4.5	0.6	7.0	0.1
20E2B-3-D6-IGFR	1677	DSVGDNFYQLLESLVGGHGVG	20.7	17.8	2.6	6.9	0.1
B6Hα-2-C7-IR	1678	RGIVAMVEATEVGSDHDPFYHKLSELVQGS	45.1	6.7	1.0	6.7	0.1
20E2B-2-B7-IGFR	1679	LSSDGQFYRALNLLLQGSAGR	18.0	6.1	0.9	6.7	0.1
20E2B-3-C4-IGFR	1680	ASSASGFYELLQRLAGLGLEV	23.4	20.4	3.3	6.2	0.2
20C-3-E4-IGFR	1681	FFYRCLSRLLGGQLGSRLGLSCIGD	37.7	7.7	1.3	6.0	0.2
NNRPγ-4-A1-IR	1682	IIGGFYSYFNSVLRLGT	9.7	10.9	1.8	6.0	0.2
20E2B-4-H8-IGFR	1683	PAGPCGFYCGLGLLLHGDQSP	7.2	5.3	0.9	5.9	0.2
20E2B-4-H9-IGFR	1684	RCQGTGFYTCIQELIGFGDPD	4.5	5.2	0.9	5.6	0.2
B6Hα-2-C10-IR	1685	SGAKVIVVTGDSGDPFYHKLSELLQGS	46.9	5.8	1.1	5.3	0.2
20E2A-3-C7-IGFR	1686	VGTVAGFYDAIAQLVARASRV	17.6	5.4	1.1	5.1	0.2
20E2B-1-A8-IGFR	1687	TLRSPTFYDWLEMVLTHGQGG	16.1	4.4	0.9	5.0	0.2
NNRPγ-4-A7-IR	1688	RFDPFYSYFVNLLGASA	2.5	6.3	1.3	4.9	0.2

			Ratios over Background			Comparisons	
Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
B6Hα-3-E8-IR	1689	RGKTAAVIVGRPADPFYHKLSELLQGG	47.6	5.3	1.1	4.8	0.2
B6Hα-3-F10-IR	1690	GCVVEWQKWHGASDPFYHKLSELGGCS	47.2	8.8	1.9	4.6	0.2
B6Hα-2-D6-IR	1691	GRTMAVMAAGGPDDPFYHKLSELVQGG	33.5	4.4	1.0	4.4	0.2
B6Hα-3-E7-IR	1692	GCAVVEEAERSRGDPFYHKLSELIQGC	47.0	5.6	1.3	4.3	0.2
B6Hα-2-D1-IR	1693	GCEVIVEEGDSADPFYHKLSELCQGS	11.7	5.4	1.3	4.2	0.2
20E2A-3-D10-IGFR	1694	MMVVDGFYDALHQLVVAQSLG	20.6	6.9	1.8	3.9	0.3
20E2A-3-A12-IGFR	1695	LSVALSFYDALGQLVAGEGRW	16.1	4.3	1.1	3.9	0.3
B6Hα-4-G8-IR	1696	GGTKAVAKVGTRDDPFYHKLSELLQGS	32.3	6.1	1.7	3.6	0.3
B6L-4-D7-IR	1697	AETSVQVGWIRLQSVWPGEHWNTVDPFYHKLSELLRGSGA	14.3	4.8	1.4	3.4	0.3
B6Hα-1-A3-IR	1698	SRAKVEAEMPDSGDPFYHKLSELLASG	37.4	2.6	0.8	3.3	0.3
B6Hα-3-F7-IR	1699	SRVAATKEKRPSDDPFYHKLSELLQGS	41.5	3.1	1.0	3.1	0.3
B6Hα-2-D8-IR	1700	SSETAKMVTGTRDDPFYHKLSELVQGS	19.3	3.0	1.0	3.0	0.3
B6Hα-1-B3-IR	1701	GCITAENGAGDPFYHKLSELGGCS	33.1	3.2	1.1	2.9	0.3
B6Hα-3-E5-IR	1702	RCGDEEGWQENRRDDPFYHKLSELFGGC	28.8	2.9	1.0	2.9	0.3
20E2A-4-G11-IGFR	1703	MNVFVSFYDAIDQLVCQRIGC	20.7	3.3	1.3	2.6	0.4
20E2Bβ-3-C7-IR	1704	QSGSGDFYDWLSRLIRGNGDG	1.5	3.1	1.5	2.0	0.5
B6Hα-3-E6-IR	1705	CGAKMTGTPNDPFYHKLSELLQRG	18.2	2.3	1.2	1.9	0.5
20E2A-3-A3-IGFR	1706	GHYFGSFYDAIDQLVAGMLPG	5.2	3.0	1.5	1.9	0.5
B6L-4-A7-IR	1707	AGTPAQVG*NRLWSVWPGEHWNTVDPFYNKLSELLRESGA	11.6	3.4	1.9	1.8	0.6
B6Hα-3-F1-IR	1708	CSMAAVAEAGDDDDPFYHKLSELCQGS	22.5	2.4	1.3	1.8	0.5
B6L-3-G6-IR	1709	VDTPAQVGWNRLWSVGPGEHWYTDDPFYH*LSELLRESGA	7.6	2.5	1.8	1.4	0.7
B6L-3-G5-IR	1710	AETSAQVGWQRLWSVWPGDHWSTLDPFYHKLSELLRESGA	11.5	2.0	1.4	1.4	0.7
20E2A-3-A4-IGFR	1711	AGSVTSFYDAMEQLVATGTSA	16.8	2.5	1.8	1.4	0.7
B6-PD1-IGFR	1712	TDDGFYDALEQLVQGSKK					
20E2-PD1-IGFR(RP10)	1713	GSFYEALQRLVGGEQGKK					

FORMULA 10 (Group 6):

			Ratios over Background			Comparisons	
Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
R20β-4-E8-IR	1714	VRGFQGGTVWPGYEWLRNAA	41.0	34.9	3.6	9.7	0.1
40F-4-D1-IGFR	1715	LSCLAYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE	4.9	4.6	0.3	13.1	0.1
40F-4-B1-IGFR	1716	GLDHSDAVGVHLGFAWPAQARGRWEAGGLEDTWAGYDWL	4.1	3.0	0.2	13.1	0.1
40F-4-D10-IGFR	1717	W.GYAWLS	4.9	4.5	0.4	11.7	0.1

Besides relative binding at IR or IGF-1R, relative efficacy at the cognate receptor is another important consideration for choosing a potential therapeutic. Thus, a sequence that is efficacious at IR but has little or no significant activity at IGF-1R may also be considered as an important IR therapeutic, irrespective of the relative binding affinities at IR and IGF-1R. For example, A6 selectivity for IR may be enhanced by including glutamic acid in a carboxyl terminal extension at position X_{95}. IR selectivity of the B6 motif may be enhanced by having a tryptophan or phenylalanine at X_{11}. Tryptophan at X_{13} also favors selectivity of IR. A tryptophan amino acid at X_{13} rather than leucine at that position also may be used to enhance selectivity for IR. In the reverse B6 motif, a large amino acid at X_{15} favors IR selectivity. Conversely, small amino acids may confer specificity for IGF-1R. In the F8 motif, an L in position X_{23} is essentially required for IR binding. In addition, tryptophan at X_{31} is also highly preferred. At X_{32}, glycine is preferred for IR selectivity.

D. Multiple Binding Sites On IR And IGF-1R

The competition data disclosed herein reveals that at least two separate binding sites are present on IR and IGF-1R which recognize the different sequence motifs provided by this invention.

As shown in Figure 6, competition data indicate that peptides comprising the A6 motifs compete for binding to the same site on IR (Site 1) whereas the D8 motifs compete for a second site (Site 2). The identification of peptides that bind to separate binding sites on IR and IGF-1R provides for various schemes of binding to IR or IGF-1R to increase or decrease its activity. Examples of such schemes for IR are illustrated in Figure 7.

The table below shows sequences based on their groups, which bind to Site 1 or Site 2.

TABLE 2

REPRESENTATIVE SITE 1 PEPTIDES

A6-like (FYxWF) (SEQ ID NO:1596):

Clone	Sequence	SEQ ID NO:
G3	KRGGGTFYEWFESALRKHGAGKK	1718
H2	VTFTSAVFHENFYDWFVRQVSKK	1719
H2C	**FHENFYDWFVRQVSKK**	**1556**
A6S-IR3-E12	**GRVDWLQRNANFYDWFVAELG**	**1560**
A6S-IR4-G1	**NGVERAGTGDNFYDWFVAQLH**	**1720**
H2CB-R3-B12	**QSDSGTVHDRFYGWFRDTWAS**	**1721**
20E2A-R3-B11	GRFYGWFQDAIDQLMPWGFDP	1722
rB6-F6	RYGRWGLAQQFYDWFDR	1723
E4Dα-1-B8-IR~	GFREGQRWYWFVAQVT	1724
H2CA-4-F11-IR	TYKARFLHENFYDWFNRQVSQYFGRV	1725
H2CB-R3-D2	WTDVDGFHSGFYRWFQNQWER	1726
H2CB-R3-D12	VASGHVLHGQFYRWFVDQFAL	1727
H2CB-R4-H5	QARVGNVHQQFYEWFREVMQG	1728
H2C-B-E8*	TGHRLGLDEQFYWWFRDALSG	1729
H2CB-3-B6-IR~	VGDFCVSHDCFYGWFLRESMQ	1730
A6S-IR2-C1	RMYFSTGAPQNFYDWFVQEWD	1731

B6-like (FYxxLxxL) (SEQ ID NO:1732):

Clone	Sequence	
20C11	**KDRAFYNGLRDLVGAVYGAWDKK**	**1733**
20E2	**DYKDFYDAIDQLVRGSARAGGTRDKK**	**1734**
B62-R3-C7	**EHWNTVDPFYFTLFEWLRESG**	**1735**
B62-R3-C10	**EHWNTVDPFYQYFSELLRESG**	**1736**
20E2B-3-B3-IR	AGVNAGFYRYFSTLLDWWDQG	1737
20E2-B-E3*	IQGWEPFYGWFDDVVAQMFEE	1738
20E2A-R4-F9	PPWGARFYDAIEQLVFDNLCC	1739
RPNN-4-G6-HOLO*	RWPNFYGYFESLLTHFS	1740
RPNN-4-F3-HOLO*	HYNAFYEYFQVLLAETW	1741
20E2A-R4-E2	IGRVRSFYDAIDKLFQSDWER	1742
RPNN-2-C1-IR*	EGWDFYSYFSGLLASVT	1743
20E2B-4-F12-IR	SVKEVQFYRYFYDLLQSEESG	1744
20E2-B-E12	GNSGGSFYRYFQLLLDSDGMS	1745
20E2A-R3-B6	RDAGSSFYDAIDQLVCLTYFC	1746

Reverse B6-like (LxxLxxYF) (SEQ ID NO:1747):

Clone	Sequence	
rB6-A12	**LDALDRLMRYFEERPSL**	**1748**
rB6-F9	**PLAELWAYFEHSEQGRSSAH**	**1749**
rB6-4-E7-IR	LDPLDALLQYFWSVPGH	1750
rB6-4-F9-IR	RGRLGSLSTQFYNWFAE	1751
rB6-E6	ADELEWLLDYFMHQPRP	1752
rB6-4-F12-IR	DGVLEELFSYFSATVGP	1753

Group 6 (WPxYxWL) (SEQ ID NO:1754):

Clone	Sequence	
R20β-4-A4-IR	WPGYLFFEEALQDWRGSTED	1755

Peptides by design**:

Clone	Sequence	
H2C-PD1-IR~	AAVHEQFYDWFADQYKK	1756
A6S-PD1-IR~	QAPSNFYDWFVREWDKK	1757

20E2-PD1-IR~	QSFYDYIEELLGGEWKK	1758
B6C-PD1-IR~	DPFYQGLWEWLRESGKK	1759

REPRESENTATIVE SITE 2 PEPTIDES (C-C LOOPS)

F8-derived (Long C-C loop):

Clone	Sequence	SEQ ID NO:
F8	HLCVLEELFWGASLFGYCSG	1760
F8-C12	**FQSLLEELVWGAPLFRYGTG**	**1761**
F8-Des2	**PLCVLEELFWGASLFGYCSG**	**1762**
F8-F12	PLCVLEELFWGASLFGQCSG	1763
F8-B9	HLCVLEELFWGASLFGQCSG	1764
F8-B12	DLRVLCELFGGAYVLGYCSE	1765
NNKH-2B3	HRSVLKQLSWGASLFGQWAG	1766
NNKH-2F9~	HLSVGEELSWWVALLGQWAR	1767
NNKH-4H4~	APVSTEELRWGALLFGQWAG	1768

D8-derived (Small C-C loop):

Clone	Sequence	SEQ ID NO:
D8	**KWLDQEWAWVQCEVYGRGCPSKK**	**1769**
D8-G1	**QLEEEWAGVQCEVYGRECPS**	**1770**
D8-B5~	ALEEEWAWVQVRSIRSGLPL	1771
D8-A7	SLDQEWAWVQCEVYGRGCLS	1772
D8-F1~	WLEHEWAQIQCELYGRGCTY	1773

Midi C-C loop:

Clone	Sequence	
D8-F10	GLEQGCPWVGLEVQCRGCPS	1774
F8-B12~	DLRVLCELFGGAYVLGYCSE	1775
F8-A9	PLWGLCELFGGASLFGYCSS	1776

**Based on analysis of entire panning data, amino acid preferences at each position were calculated to define these "idealized" peptides; * Peptides synthesized and currently being purified; ~ Peptides planned.

In various aspects of the present invention, amino acid sequences comprising Site 1 motifs may bind to Site 1 of IR or Site 1 of IGF-1R. Similarly, amino acids sequences comprising Site 2 motifs may bind to Site 2 of IR or Site 2 of IGF-1R. However, specific peptides may show higher binding affinity for IR than for IGF-1R, while other peptides may show higher binding affinity for IGF-1R than for IR. In addition, Site 1 and Site 2 on IR do not cross-talk, i.e., Site 1-binding sequences do not compete with Site 2-binding sequences at IR. In contrast, Site 1 and Site 2 on IGF-1R do show some cross-talk, suggesting an allosteric effect. These aspects are illustrated in the Examples described hereinbelow.

E. Multivalent Ligands

This invention provides ligands that preferentially bind different sites on IR and IGF-1R. The A6 amino acid sequence motif confers binding to IR at Site 1 (Figure 6). The D8 amino acid sequence motif confers binding to IR at Site 2 (Figure 6). Accordingly, multimeric ligands may be prepared according to the invention by covalently linking amino acid sequences. Depending on the purpose intended for the multivalent ligand, amino acid sequences that bind the same or different sites may be combined to form a single molecule. Where the multivalent ligand is constructed to bind to the same corresponding site on different receptors, or different subunits of a receptor, the amino acid sequences of the ligand for binding to the receptors may be the same or different, provided that if different amino acid sequences are used, they both bind to the same site.

Multivalent ligands may be prepared by either expressing amino acid sequences which bind to the individual sites separately and then covalently linking them together, or by expressing the multivalent ligand as a single amino acid sequence which comprises within it the combination of specific amino acid sequences for binding.

Various combinations of amino acid sequences may be combined to produce multivalent ligands having specific desirable properties. Thus, agonists may be combined with agonists, antagonists combined with antagonists, and agonists combined with antagonists. Combining amino acid sequences that bind to the same site to form a multivalent ligand may be useful to produce molecules that are capable of cross-linking together multiple receptor units. Multivalent ligands may also be constructed to combine amino acid sequences which bind to different sites (Figure 7).

In view of the discovery disclosed herein of monomers having agonist properties at IR or IGF-1R, preparation of multivalent ligands may be useful to prepare ligands having more desirable pharmacokinetic properties due to the presence of multiple bind sites on a single molecule. In addition,

combining amino acid sequences that bind to different sites with different affinities provides a means for modulating the overall potency and affinity of the ligand for IR or IGF-1R.

1. Construction of Hybrids

In one embodiment, hybrids of at least two peptides (e.g., dimer peptides) may be produced as recombinant fusion polypeptides, which are expressed in any suitable expression system. The polypeptides may bind the receptor as either fusion constructs containing amino acid sequences besides the ligand binding sequences or as cleaved proteins from which signal sequences or other sequences unrelated to ligand binding are removed. Sequences for facilitating purification of the fusion protein may also be expressed as part of the construct. Such sequences optionally may be subsequently removed to produce the mature binding ligand. Recombinant expression also provides means for producing large quantities of ligand. In addition, recombinant expression may be used to express different combinations of amino acid sequences and to vary the orientation of their combination, i.e., amino to carboxyl terminal orientation.

In one embodiment shown below (Figure 28), MBP-FLAG®-PEPTIDE-$(GGS)_n$ (SEQ ID NO:1777)-PEPTIDE-E-TAG, a fusion construct producing a peptide dimer comprises a maltose binding protein amino acid sequence (MBP) or similar sequence useful for enabling the affinity chromatography purification of the expressed peptide sequences. This purification facilitating sequence may then be attached to a FLAG® sequence to provide a cleavage site to remove the initial sequence. The dimer then follows which includes the intervening linker and a tag sequence may be included at the carboxyl terminal portion to facilitate identification/purification of the expression of peptide. In the representative construct illustrated above, G and S are glycine and serine residues, which make up the linker sequence. As non-limiting examples, n can be 1, 2, 3, or 4 to yield a linker sequence of 3, 6, 9, and 12 amino acids, respectively.

In addition to producing the dimer peptides by recombinant protein expression, dimer peptides may also be produced by peptide synthesis whereby a synthetic technique such as Merrifield synthesis (Merrifield, 1997), may be used to construct the entire peptide.

Other methods of constructing dimer peptides include introducing a linker molecule that activates the terminal end of a peptide so that it can covalently bind to a second peptide. Examples of such linkers include, but are not limited to, diaminoproprionic acid activated with an oxyamino function. A preferred linker is a dialdehyde having the formula O=CH-$(CH_2)_n$-CH=O, wherein n is at least 2 to 6, but is preferably 6 to produce a linker of about 25 to 30 angstroms in length. Other preferred linkers are shown in Table 3. Linkers may be used, for example, to couple monomers at either the carboxyl terminal or the amino terminal ends to form dimer peptides. Also, the chemistry can be inverted, i.e., the peptides to be coupled can be equipped with aldehyde functions, either by oxidation with sodium periodate of an N-terminal serine, or by oxidation of any other vicinal hydroxy- or amino-groups, and the linker can comprise two oxyamino functions (e.g., at end of a polyethylene glycol linker) or amino groups which are coupled by reductive amination.

In specific embodiments, Site 1-Site 2 and Site 2-Site 1 orientations are possible. In addition, N-terminal to N-terminal (N-N); C-terminal to C-terminal (C-C); N-terminal to C-terminal (N-C); and C-terminal to N-terminal (C-N) linkages are possible. Accordingly, peptides may be oriented Site 1 to Site 2, or Site 2 to Site 1, and may be linked N-terminus to N-terminus, C-terminus to C-terminus, N-terminus to C-terminus, or C-terminus to N-terminus. In certain cases, a specific orientation may be preferable to others, for example, for maximal agonist or antagonist activity.

In an unexpected and surprising result, the orientation and linkage of the monomer subunits has been found to dramatically alter dimer activity (see Examples, below). In particular, certain Site 1/Site 2 heterodimer sequences show agonist or antagonist activity at IR, depending on

orientation and linkage of the constituent monomer subunits. For example, a Site 1-Site 2 orientation (C-N linkage), e.g., the S453 heterodimer, shows antagonist activity at IR (Figure 18A; Table 7). In contrast, a Site 2-Site 1 orientation (C-N linkage), e.g., the S455 heterodimer, shows potent agonist activity at IR (Figure 18D; Table 7). Similarly, Site 1-Site 2 (C-N linkage) heterodimers, e.g., S425 and S459, show antagonist activity at IR (Table 7), while Site 1-Site 2 (C-C or N-N linkage) heterodimers, e.g., S432-S438, S454, and S456, show agonist activity (Table 7).

Whether produced by recombinant gene expression or by conventional chemical linkage technology, the various amino acid sequences may be coupled through linkers of various lengths. Where linked sequences are expressed recombinantly, and based on an average amino acid length of about 4 angstroms, the linkers for connecting the two amino acid sequences would typically range from about 3 to about 12 amino acids corresponding to from about 12 to about 48 Å. Accordingly, the preferred distance between binding sequences is from about 2 to about 50 Å. More preferred is 4 to about 40. The degree of flexibility of the linker between the amino acid sequences may be modulated by the choice of amino acids used to construct the linker. The combination of glycine and serine is useful for producing a flexible, relatively unrestrictive linker. A more rigid linker may be constructed by using amino acids with more complex side chains within the linkage sequence.

2. Characterization Of Specific Dimers

Specific dimers which are comprised of monomer subunits that both bind with high affinity to the same site on IR or IGF-1R (e.g., Site 1-Site 1 or Site 2-Site 2), or monomer subunits that bind to different sites on IR or IGF-1R (e.g., Site 1-Site 2 or Site 2-Site 1) are disclosed herein.

Other combinations of peptides are within the scope of this invention and may be determined as demonstrated in the examples described herein.

F. Peptide Synthesis

Many conventional techniques in molecular biology, protein biochemistry, and immunology may be used to produce the amino acid sequences for use with this invention. The present invention encompasses the specific amino acid sequences shown in Figures 1-4, 8, and 9 and Table 7, *inter alia*, without additions (e.g., linker or spacer sequences) deletions, alterations, or modification. The present invention further encompasses variants that include additional sequences, altered sequences, and functional fragments thereof. In a preferred embodiment, the amino acid sequence variant or fragment shares at least one function characteristic (e.g., binding, agonist, or antagonist activity) of the reference sequence. Variant peptides include, for example, genetically engineered mutants, and may differ from the amino acid sequences shown in the figures and tables of the application by the addition, deletion, or substitution of one or more amino acid residues. Alterations may occur at the amino- or carboxy-terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. In addition, variants may comprise synthetic or non-naturally occurring amino acids in accordance with this invention.

Variant amino acid sequences can have conservative changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More infrequently, a variant peptide can have non-conservative changes, e.g., substitution of a glycine with a tryptophan. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing binding or biological activity can be found using computer programs well-known in the art, for example, DNASTAR software (DNASTAR, Inc., Madison, WI). Guidance is also provided by the data disclosed herein. In particular, Figures 1-4, 8, 9, 43, 44, and Table 7, *inter alia*, teach which amino acid

residues can be deleted, added, substituted, or modified, while maintaining the IR- or IGF-1R-related function(s) (e.g., binding, agonist, or antagonist activity) of the amino acid sequences.

For the purposes of this invention, the amino acids are grouped as follows: amino acids possessing alcohol groups are serine (S) and threonine (T). Aliphatic amino acids are isoleucine (I), leucine (L), valine (V), and methionine (M). Aromatic amino acids are phenylalanine (F), histidine (H), tryptophan (W), and tyrosine (Y). Hydrophobic amino acids are alanine (A), cysteine (C), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), leucine (L), methionine (M), arginine (R), threonine (T), valine (V), tryptophan (W), and tyrosine (Y). Negative amino acids are aspartic acid (D) and glutamic acid (E). The following amino acids are polar amino acids: cysteine (C), aspartic acid (D), glutamic acid (E), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), and threonine (T). Positive amino acids are histidine (H), lysine (K), and arginine (R). Small amino acids are alanine (A), cysteine (C), aspartic acid (D), glycine (G), asparagine (N), proline (P), serine (S), threonine (T), and valine (V). Very small amino acids are alanine (A), glycine (G) and serine (S). Amino acids likely to be involved in a turn formation are alanine (A), cysteine (C), aspartic acid (D), glutamic acid (E), glycine (G), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), proline (P), and threonine (T). As non-limiting examples, the amino acids within each of these defined groups may be substituted for each other in the formulas described above, as conservative substitutions, subject to the specific preferences stated herein.

Substantial changes in function can be made by selecting substitutions that are less conservative than those shown in the defined groups, above. For example, non-conservative substitutions can be made which more significantly affect the structure of the peptide in the area of the alteration, for example, the alpha-helical, or beta-sheet structure; the charge or hydrophobicity of the molecule at the target site; or the bulk of the side

chain. The substitutions which generally are expected to produce the greatest changes in the peptide's properties are those where 1) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl, or alanyl; 2) a cysteine or proline is substituted for (or by) any other residue; 3) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or 4) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) a residue that does not have a side chain, e.g., glycine.

Amino acid preferences have been identified for certain peptides and peptide groups of the present invention. For example, amino acid preferences for the RP9, D8, and Group 6 (Formula 10) peptides are shown in Tables 17-19, below. In some instances, cysteine pairs may also be preferred. For example, cysteine pairs are preferred in certain Formula 1 and Formula 2 sequences described herein. In accordance with the invention, the amino acid sequences of the invention may include two or more cysteine residues, which may be separated by at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 amino acids, and may be positioned inside or outside the Formula 1 or Formula 2 motif sequence. Preferably, the cysteines are separated by 17 or 18 amino acids.

Variants also include amino acid sequences in which one or more residues are modified (i.e., by phosphorylation, sulfation, acylation, PEGylation, etc.), and mutants comprising one or more modified residues. Amino acid sequences may also be modified with a label capable of providing a detectable signal, either directly or indirectly, including, but not limited to, radioisotope, fluorescent, and enzyme labels. Fluorescent labels include, for example, Cy3, Cy5, Alexa, BODIPY, fluorescein (e.g., FluorX, DTAF, and FITC), rhodamine (e.g., TRITC), auramine, Texas Red, AMCA blue, and Lucifer Yellow. Preferred isotope labels include ^{3}H, ^{14}C, 32 P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re. Preferred enzyme labels include peroxidase, β-glucuronidase, β-D-glucosidase, β-D-

galactosidase, urease, glucose oxidase plus peroxidase, and alkaline phosphatase (see, e.g., U.S. Pat. Nos. 3,654,090; 3,850,752 and 4,016,043). Enzymes can be conjugated by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde, and the like. Enzyme labels can be detected visually, or measured by calorimetric, spectrophotometric, fluorospectrophotometric, amperometric, or gasometric techniques. Other labeling systems, such as avidin/biotin, Tyramide Signal Amplification (TSA™), are known in the art, and are commercially available (see, e.g., ABC kit, Vector Laboratories, Inc., Burlingame, CA; NEN® Life Science Products, Inc., Boston, MA).

1. Recombinant Synthesis of Peptides

To obtain recombinant peptides, DNA sequences encoding these peptides may be cloned into any suitable vectors for expression in intact host cells or in cell-free translation systems by methods well-known in the art (see Sambrook *et al.*, 1989). The particular choice of the vector, host, or translation system is not critical to the practice of the invention.

A large number of vectors, including bacterial, yeast, and mammalian vectors, have been described for replication and/or expression in various host cells or cell-free systems, and may be used for gene therapy as well as for simple cloning or protein expression. In one aspect of the present invention, an expression vector comprises a nucleic acid encoding a IR or IGF-1R agonist or antagonist peptide, as described herein, operably linked to at least one regulatory sequence. Regulatory sequences are known in the art and are selected to direct expression of the desired protein in an appropriate host cell. Accordingly, the term regulatory sequence includes promoters, enhancers and other expression control elements (see D.V. Goeddel (1990) *Methods Enzymol.* **185**:3-7). Enhancer and other expression control sequences are described in *Enhancers and Eukaryotic Gene Expression*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1983). It should be understood that the design of the expression vector

may depend on such factors as the choice of the host cell to be transfected and/or the type of peptide desired to be expressed.

Several regulatory elements (e.g., promoters) have been isolated and shown to be effective in the transcription and translation of heterologous proteins in the various hosts. Such regulatory regions, methods of isolation, manner of manipulation, etc. are known in the art. Non-limiting examples of bacterial promoters include the β-lactamase (penicillinase) promoter; lactose promoter; tryptophan (trp) promoter; araBAD (arabinose) operon promoter; lambda-derived P_1 promoter and N gene ribosome binding site; and the hybrid tac promoter derived from sequences of the trp and lac UV5 promoters. Non-limiting examples of yeast promoters include the 3-phosphoglycerate kinase promoter, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter, galactokinase (GAL1) promoter, galactoepimerase promoter, and alcohol dehydrogenase (ADH1) promoter. Suitable promoters for mammalian cells include, without limitation, viral promoters, such as those from Simian Virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus (ADV), and bovine papilloma virus (BPV). Preferred replication and inheritance systems include M13, ColE1, SV40, baculovirus, lambda, adenovirus, CEN ARS, 2μm ARS and the like. While expression vectors may replicate autonomously, they may also replicate by being inserted into the genome of the host cell, by methods well-known in the art.

To obtain expression in eukaryotic cells, terminator sequences, polyadenylation sequences, and enhancer sequences that modulate gene expression may be required. Sequences that cause amplification of the gene may also be desirable. Furthermore, sequences that facilitate secretion of the recombinant product from cells, including, but not limited to, bacteria, yeast, and animal cells, such as secretory signal sequences and/or preprotein or proprotein sequences, may also be included. These sequences are well-described in the art. DNA sequences can be optimized, if desired, for more efficient expression in a given host organism or expression system. For example, codons can be altered to conform to the

preferred codon usage in a given host cell or cell-free translation system using well-established techniques.

Codon usage data can be obtained from publicly-available sources, for example, the Codon Usage Database at http://www.kazusa.or.jp/codon/. In addition, computer programs that translate amino acid sequence information into nucleotide sequence information in accordance with codon preferences (i.e., backtranslation programs) are widely available. See, for example, Backtranslate program from Genetics Computer Group (GCG), Accelrys, Inc., Madison, WI; and Backtranslation Applet from Entelechon GmbH, Regensburg, Germany. Thus, using the peptide sequences disclosed herein, one of ordinary skill in the art can design nucleic acids to yield optimal expression levels in the translation system or host cell of choice.

Expression and cloning vectors will likely contain a selectable marker, a gene encoding a protein necessary for survival or growth of a host cell transformed with the vector. The presence of this gene ensures growth of only those host cells that express the inserts. Typical selection genes encode proteins that 1) confer resistance to antibiotics or other toxic substances, e.g., ampicillin, neomycin, methotrexate, etc.; 2) complement auxotrophic deficiencies, or 3) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*. Markers may be an inducible or non-inducible gene and will generally allow for positive selection. Non-limiting examples of markers include the ampicillin resistance marker (i.e., beta-lactamase), tetracycline resistance marker, neomycin/kanamycin resistance marker (i.e., neomycin phosphotransferase), dihydrofolate reductase, glutamine synthetase, and the like. The choice of the proper selectable marker will depend on the host cell, and appropriate markers for different hosts as understood by those of skill in the art.

Suitable expression vectors for use with the present invention include, but are not limited to, pUC, pBluescript (Stratagene), pET

(Novagen, Inc., Madison, WI), and pREP (Invitrogen) plasmids. Vectors can contain one or more replication and inheritance systems for cloning or expression, one or more markers for selection in the host, e.g., antibiotic resistance, and one or more expression cassettes. The inserted coding sequences can be synthesized by standard methods, isolated from natural sources, or prepared as hybrids. Ligation of the coding sequences to transcriptional regulatory elements (e.g., promoters, enhancers, and/or insulators) and/or to other amino acid encoding sequences can be carried out using established methods.

Suitable cell-free expression systems for use with the present invention include, without limitation, rabbit reticulocyte lysate, wheat germ extract, canine pancreatic microsomal membranes, *E. coli* S30 extract, and coupled transcription/translation systems (Promega Corp., Madison, WI). These systems allow the expression of recombinant peptides upon the addition of cloning vectors, DNA fragments, or RNA sequences containing protein-coding regions and appropriate promoter elements.

Non-limiting examples of suitable host cells include bacteria, archea, insect, fungi (e.g., yeast), plant, and animal cells (e.g., mammalian, especially human). Of particular interest are *Escherichia coli, Bacillus subtilis, Saccharomyces cerevisiae,* SF9 cells, C129 cells, 293 cells, *Neurospora*, and immortalized mammalian myeloid and lymphoid cell lines. Techniques for the propagation of mammalian cells in culture are well-known (see, Jakoby and Pastan (Eds), 1979, *Cell Culture. Methods in Enzymology*, volume 58, Academic Press, Inc., Harcourt Brace Jovanovich, NY). Examples of commonly used mammalian host cell lines are VERO and HeLa cells, CHO cells, and WI38, BHK, and COS cell lines, although it will be appreciated by the skilled practitioner that other cell lines may be used, e.g., to provide higher expression, or other features.

Host cells can be transformed, transfected, or infected as appropriate by any suitable method including electroporation, calcium chloride-, lithium chloride-, lithium acetate/polyethylene glycol-, calcium phosphate-, DEAE-

dextran-, liposome-mediated DNA uptake, spheroplasting, injection, microinjection, microprojectile bombardment, phage infection, viral infection, or other established methods. Alternatively, vectors containing the nucleic acids of interest can be transcribed *in vitro*, and the resulting RNA introduced into the host cell by well-known methods, e.g., by injection (see, Kubo *et al.*, 1988, *FEBS Letts.* **241**:119). The cells into which have been introduced nucleic acids described above are meant to also include the progeny of such cells.

Nucleic acids encoding the peptides of the invention may be isolated directly from recombinant phage libraries (e.g., RAPIDLIB® or GRABLIB® libraries) described herein. Alternatively, the polymerase chain reaction (PCR) method can be used to produce nucleic acids of the invention, using the recombinant phage libraries as templates. Primers used for PCR can be synthesized using the sequence information provided herein and can further be designed to introduce appropriate new restriction sites, if desirable, to facilitate incorporation into a given vector for recombinant expression.

Nucleic acids encoding the peptides of the present invention can also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage *et al.*, 1981, *Tetra. Letts.* **22**:1859-1862, or the triester method according to Matteucci *et al.*, 1981, *J. Am. Chem. Soc.*, **103**:3185, and can performed on commercial, automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single-stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strands together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

The nucleic acids encoding the peptides of the invention can be produced in large quantities by replication in a suitable host cell. Natural or synthetic nucleic acid fragments, comprising at least ten contiguous bases coding for a desired amino acid sequence can be incorporated into recombinant nucleic acid constructs, usually DNA constructs, capable of

introduction into and replication in a prokaryotic or eukaryotic cell. Usually the nucleic acid constructs will be suitable for replication in a unicellular host, such as yeast or bacteria, but may also be intended for introduction to (with and without integration within the genome) cultured mammalian or plant or other eukaryotic cells, cell lines, tissues, or organisms. The purification of nucleic acids produced by the methods of the present invention is described, for example, in Sambrook *et al.*, 1989; F.M. Ausubel *et al.*, 1992, *Current Protocols in Molecular Biology*, J. Wiley and Sons, New York, NY.

These nucleic acids can encode variant or truncated forms of the peptides as well as the reference peptides shown in Figures 1-4, 8, and 9 and Table 7, *inter alia*. Large quantities of the nucleic acids and peptides of the present invention may be prepared by expressing the nucleic acids or portions thereof in vectors or other expression vehicles in compatible prokaryotic or eukaryotic host cells. The most commonly used prokaryotic hosts are strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used. Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, or amphibian or avian species, may also be useful for production of the proteins of the present invention. For example, insect cell systems (i.e., lepidopteran host cells and baculovirus expression vectors) are particularly suited for large-scale protein production.

Host cells carrying an expression vector (i.e., transformants or clones) are selected using markers depending on the mode of the vector construction. The marker may be on the same or a different DNA molecule, preferably the same DNA molecule. In prokaryotic hosts, the transformant may be selected, e.g., by resistance to ampicillin, tetracycline or other antibiotics. Production of a particular product based on temperature sensitivity may also serve as an appropriate marker.

For some purposes, it is preferable to produce the peptide in a recombinant system in which the peptide contains an additional sequence

(e.g., epitope or protein) tag that facilitates purification. Non-limiting examples of epitope tags include c-myc, haemagglutinin (HA), polyhistidine (6X-HIS)(SEQ ID NO:1778), GLU-GLU, and DYKDDDDK (SEQ ID NO:1779) or DYKD (SEQ ID NO:1545; FLAG®) epitope tags. Non-limiting examples of protein tags include glutathione-S-transferase (GST), green fluorescent protein (GFP), and maltose binding protein (MBP). In one approach, the coding sequence of a peptide can be cloned into a vector that creates a fusion with a sequence tag of interest. Suitable vectors include, without limitation, pRSET (Invitrogen Corp., San Diego, CA), pGEX (Amersham Pharmacia Biotech, Inc., Piscataway, NJ), pEGFP (CLONTECH Laboratories, Inc., Palo Alto, CA), and pMAL™ (New England BioLabs, Inc., Beverly, MA) plasmids. Following expression, the epitope or protein tagged peptide can be purified from a crude lysate of the translation system or host cell by chromatography on an appropriate solid-phase matrix. In some cases, it may be preferable to remove the epitope or protein tag (i.e., via protease cleavage) following purification.

Methods for directly purifying peptides from sources such as cellular or extracellular lysates are well-known in the art (see Harris and Angal, 1989). Such methods include, without limitation, sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), preparative disc-gel electrophoresis, isoelectric focusing, high-performance liquid chromatography (HPLC), reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, countercurrent distribution, and combinations thereof. Peptides can be purified from many possible sources, for example, plasma, body tissues, or body fluid lysates derived from human or animal, including mammalian, bird, fish, and insect sources.

Antibody-based methods may also be used to purify peptides. Antibodies that recognize these peptides or fragments derived therefrom can be produced and isolated. The peptide can then be purified from a crude lysate by chromatography on an antibody-conjugated solid-phase matrix (see Harlow and Lane, 1998).

2. Chemical Synthesis Of Peptides

Alternately, peptides may be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. The peptides are preferably prepared by solid-phase peptide synthesis; for example, as described by Merrifield (1965; 1997).

According to methods known in the art, peptides can be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation, classical solution synthesis. In addition, recombinant and synthetic methods of peptide production can be combined to produce semi-synthetic peptides. The peptides of the invention are preferably prepared by solid phase peptide synthesis as described by Merrifield, 1963, *J. Am. Chem. Soc.* **85**:2149; 1997. In one embodiment, synthesis is carried out with amino acids that are protected at the alpha-amino terminus. Trifunctional amino acids with labile side-chains are also protected with suitable groups to prevent undesired chemical reactions from occurring during the assembly of the peptides. The alpha-amino protecting group is selectively removed to allow subsequent reaction to take place at the amino-terminus. The conditions for the removal of the alpha-amino protecting group do not remove the side-chain protecting groups.

The alpha-amino protecting groups are those known to be useful in the art of stepwise peptide synthesis. Included are acyl type protecting groups, e.g., formyl, trifluoroacetyl, acetyl, aromatic urethane type protecting groups, e.g., benzyloxycarbonyl (Cbz), substituted benzyloxycarbonyl and 9-fluorenylmethyloxycarbonyl (Fmoc), aliphatic urethane protecting groups, e.g., t-butyloxycarbonyl (Boc), isopropyloxycarbonyl, cyclohexyloxycarbonyl, and alkyl type protecting groups, e.g., benzyl, triphenylmethyl. The preferred protecting group is Boc. The side-chain protecting groups for Tyr

include tetrahydropyranyl, tert-butyl, trityl, benzyl, Cbz, 4-Br-Cbz and 2,6-dichlorobenzyl. The preferred side-chain protecting group for Tyr is 2,6-dichlorobenzyl. The side-chain protecting groups for Asp include benzyl, 2,6-dichlorobenzyl, methyl, ethyl, and cyclohexyl. The preferred side-chain protecting group for Asp is cyclohexyl. The side-chain protecting groups for Thr and Ser include acetyl, benzoyl, trityl, tetrahydropyranyl, benzyl, 2,6-dichlorobenzyl, and Cbz. The preferred protecting group for Thr and Ser is benzyl. The side-chain protecting groups for Arg include nitro, Tos, Cbz, adamantyloxycarbonyl, and Boc. The preferred protecting group for Arg is Tos. The side-chain amino group of Lys can be protected with Cbz, 2-Cl-Cbz, Tos, or Boc. The 2-Cl-Cbz group is the preferred protecting group for Lys.

The side-chain protecting groups selected must remain intact during coupling and not be removed during the deprotection of the amino-terminus protecting group or during coupling conditions. The side-chain protecting groups must also be removable upon the completion of synthesis, using reaction conditions that will not alter the finished peptide.

Solid phase synthesis is usually carried out from the carboxy-terminus by coupling the alpha-amino protected (side-chain protected) amino acid to a suitable solid support. An ester linkage is formed when the attachment is made to a chloromethyl or hydroxymethyl resin, and the resulting peptide will have a free carboxyl group at the C-terminus. Alternatively, when a benzhydrylamine or p-methylbenzhydrylamine resin is used, an amide bond is formed and the resulting peptide will have a carboxamide group at the C-terminus. These resins are commercially available, and their preparation has described by Stewart *et al.*, 1984, *Solid Phase Peptide Synthesis* (2nd Edition), Pierce Chemical Co., Rockford, IL.

The C-terminal amino acid, protected at the side chain if necessary and at the alpha-amino group, is coupled to the benzhydrylamine resin using various activating agents including dicyclohexylcarbodiimide (DCC), N,N'-diisopropyl-carbodiimide and carbonyldiimidazole. Following the attachment

to the resin support, the alpha-amino protecting group is removed using trifluoroacetic acid (TFA) or HCl in dioxane at a temperature between 0 and 25°C. Dimethylsulfide is added to the TFA after the introduction of methionine (Met) to suppress possible S-alkylation. After removal of the alpha-amino protecting group, the remaining protected amino acids are coupled stepwise in the required order to obtain the desired sequence.

Various activating agents can be used for the coupling reactions including DCC,N,N'-diisopropyl-carbodiimide, benzotriazol-1-yl-oxy-tris-(dimethylamino) phosphonium hexa-fluorophosphate (BOP) and DCC-hydroxybenzotriazole (HOBt). Each protected amino acid is used in excess (>2.0 equivalents), and the couplings are usually carried out in N-methylpyrrolidone (NMP) or in DMF, CH_2Cl_2 or mixtures thereof. The extent of completion of the coupling reaction is monitored at each stage, e.g., by the ninhydrin reaction as described by Kaiser *et al.*, 1970, *Anal. Biochem.* **34**:595. In cases where incomplete coupling is found, the coupling reaction is repeated. The coupling reactions can be performed automatically with commercially available instruments.

After the entire assembly of the desired peptide, the peptide-resin is cleaved with a reagent such as liquid HF for 1-2 h at 0°C, which cleaves the peptide from the resin and removes all side-chain protecting groups. A scavenger such as anisole is usually used with the liquid HF to prevent cations formed during the cleavage from alkylating the amino acid residues present in the peptide. The peptide-resin can be deprotected with TFA/dithioethane prior to cleavage if desired.

Side-chain to side-chain cyclization on the solid support requires the use of an orthogonal protection scheme which enables selective cleavage of the side-chain functions of acidic amino acids (e.g., Asp) and the basic amino acids (e.g., Lys). The 9-fluorenylmethyl (Fm) protecting group for the side-chain of Asp and the 9-fluorenylmethyloxycarbonyl (Fmoc) protecting group for the side-chain of Lys can be used for this purpose. In these cases, the side-chain protecting groups of the Boc-protected peptide-resin

are selectively removed with piperidine in DMF. Cyclization is achieved on the solid support using various activating agents including DCC, DCC/HOBt, or BOP. The HF reaction is carried out on the cyclized peptide-resin as described above.

3. Peptide Libraries

Peptide libraries produced and screened according to the present invention are useful in providing new ligands for IR and IGF-1R. Peptide libraries can be designed and panned according to methods described in detail herein, and methods generally available to those in the art (see, e.g., U.S. Patent No. 5,723,286 issued March 3, 1998 to Dower *et al.*). In one aspect, commercially available phage display libraries can be used (e.g., RAPIDLIB® or GRABLIB®, DGI BioTechnologies, Inc., Edison, NJ; Ph.D. C7C Disulfide Constrained Peptide Library, New England Biolabs). In another aspect, an oligonucleotide library can be prepared according to methods known in the art, and inserted into an appropriate vector for peptide expression. For example, vectors encoding a bacteriophage structural protein, preferably an accessible phage protein, such as a bacteriophage coat protein, can be used. Although one skilled in the art will appreciate that a variety of bacteriophage may be employed in the present invention, in preferred embodiments the vector is, or is derived from, a filamentous bacteriophage, such as, for example, f1, fd, Pf1, M13, etc. In particular, the fd-tet vector has been extensively described in the literature (see, e.g., Zacher *et al.*, 1980, *Gene* **9**:127-140; Smith *et al.*, 1985, *Science* **228**:1315-1317; Parmley and Smith, 1988, *Gene* **73**:305-318).

The phage vector is chosen to contain or is constructed to contain a cloning site located in the 5' region of the gene encoding the bacteriophage structural protein, so that the peptide is accessible to receptors in an affinity enrichment procedure as described hereinbelow. The structural phage protein is preferably a coat protein. An example of an appropriate coat protein is pIII. A suitable vector may allow oriented cloning of the

oligonucleotide sequences that encode the peptide so that the peptide is expressed at or within a distance of about 100 amino acid residues of the N-terminus of the mature coat protein. The coat protein is typically expressed as a preprotein, having a leader sequence.

Thus, desirably the oligonucleotide library is inserted so that the N-terminus of the processed bacteriophage outer protein is the first residue of the peptide, i.e., between the 3'-terminus of the sequence encoding the leader protein and the 5'-terminus of the sequence encoding the mature protein or a portion of the 5' terminus. The library is constructed by cloning an oligonucleotide which contains the variable region of library members (and any spacers, as discussed below) into the selected cloning site. Using known recombinant DNA techniques (see generally, Sambrook *et al.*, 1989, *Molecular Cloning, A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), an oligonucleotide may be constructed which, *inter alia*; 1) removes unwanted restriction sites and adds desired ones; 2) reconstructs the correct portions of any sequences which have been removed (such as a correct signal peptidase site, for example); 3) inserts the spacer residues, if any; and/or 4) corrects the translation frame (if necessary) to produce active, infective phage.

The central portion of the oligonucleotide will generally contain one or more IR and/or IGF-1R binding sequences and, optionally, spacer sequences. The sequences are ultimately expressed as peptides (with or without spacers) fused to or in the N-terminus of the mature coat protein on the outer, accessible surface of the assembled bacteriophage particles. The size of the library will vary according to the number of variable codons, and hence the size of the peptides, which are desired. Generally the library will be at least about 10^6 members, usually at least 10^7, and typically 10^8 or more members. To generate the collection of oligonucleotides which forms a series of codons encoding a random collection of amino acids and which is ultimately cloned into the vector, a codon motif is used, such as $(NNK)_x$, where N may be A, C, G, or T (nominally equimolar), K is G or T (nominally

equimolar), and x is typically up to about 5, 6, 7, 8, or more, thereby producing libraries of penta-, hexa-, hepta-, and octa-peptides or larger. The third position may also be G or C, designated "S". Thus, NNK or NNS 1) code for all the amino acids; 2) code for only one stop codon; and 3) reduce the range of codon bias from 6:1 to 3:1.

It should be understood that, with longer peptides, the size of the library that is generated may become a constraint in the cloning process. The expression of peptides from randomly generated mixtures of oligonucleotides in appropriate recombinant vectors is known in the art (see, e.g., Oliphant *et al.*, Gene **44**:177-183). For example, the codon motif $(NNK)_6$ produces 32 codons, one for each of 12 amino acids, two for each of five amino acids, three for each of three amino acids and one (amber) stop codon. Although this motif produces a codon distribution as equitable as available with standard methods of oligonucleotide synthesis, it results in a bias against peptides containing one-codon residues. In particular, a complete collection of hexacodons contains one sequence encoding each peptide made up of only one-codon amino acids, but contains 729 (3^6) sequences encoding each peptide with only three-codon amino acids.

An alternative approach to minimize the bias against one-codon residues involves the synthesis of 20 activated trinucleotides, each representing the codon for one of the 20 genetically encoded amino acids. These are synthesized by conventional means, removed from the support while maintaining the base and 5-OH-protecting groups, and activated by the addition of 3'O-phosphoramidite (and phosphate protection with b-cyanoethyl groups) by the method used for the activation of mononucleosides (see, generally, McBride and Caruthers, 1983, *Tetrahedron Letters* **22**:245). Degenerate oligocodons are prepared using these trimers as building blocks. The trimers are mixed at the desired molar ratios and installed in the synthesizer. The ratios will usually be approximately equimolar, but may be a controlled unequal ratio to obtain the over- to under-representation of certain amino acids coded for by the

degenerate oligonucleotide collection. The condensation of the trimers to form the oligocodons is done essentially as described for conventional synthesis employing activated mononucleosides as building blocks (see, e.g., Atkinson and Smith, 1984, *Oligonucleotide Synthesis*, M.J. Gait, Ed., p. 35-82). This procedure generates a population of oligonucleotides for cloning that is capable of encoding an equal distribution (or a controlled unequal distribution) of the possible peptide sequences. Advantageously, this approach may be employed in generating longer peptide sequences, since the range of bias produced by the $(NNK)_6$ motif increases by three-fold with each additional amino acid residue.

When the codon motif is $(NNK)_x$, as defined above, and when x equals 8, there are 2.6. x 10^{10} possible octa-peptides. A library containing most of the octa-peptides may be difficult to produce. Thus, a sampling of the octa-peptides may be accomplished by constructing a subset library using up to about 10% of the possible sequences, which subset of recombinant bacteriophage particles is then screened. If desired, to extend the diversity of a subset library, the recovered phage subset may be subjected to mutagenesis and then subjected to subsequent rounds of screening. This mutagenesis step may be accomplished in two general ways: the variable region of the recovered phage may be mutagenized, or additional variable amino acids may be added to the regions adjoining the initial variable sequences.

To diversify around active peptides (i.e., binders) found in early rounds of panning, the positive phage can sequenced to determine the identity of the active peptides. Oligonucleotides can then be synthesized based on these peptide sequences. The syntheses are done with a low level of all bases incorporated at each step to produce slight variations of the primary oligonucleotide sequences. This mixture of (slightly) degenerate oligonucleotides can then be cloned into the affinity phage by methods known to those in the art. This method produces systematic, controlled variations of the starting peptide sequences as part of a secondary library. It

requires, however, that individual positive phage be sequenced before mutagenesis, and thus is useful for expanding the diversity of small numbers of recovered phage.

An alternate approach to diversify the selected phage allows the mutagenesis of a pool, or subset, of recovered phage. In accordance with this approach, phage recovered from panning are pooled and single stranded DNA is isolated. The DNA is mutagenized by treatment with, e.g., nitrous acid, formic acid, or hydrazine. These treatments produce a variety of damage to the DNA. The damaged DNA is then copied with reverse transcriptase, which misincorporates bases when it encounters a site of damage. The segment containing the sequence encoding the receptor-binding peptide is then isolated by cutting with restriction nuclease(s) specific for sites flanking the peptide coding sequence. This mutagenized segment is then recloned into undamaged vector DNA, the DNA is transformed into cells, and a secondary library according to known methods. General mutagenesis methods are known in the art (see Myers *et al.*, 1985, *Nucl. Acids Res.* **13**:3131-3145; Myers *et al.*, 1985, *Science* **229**:242-246; Myers, 1989, *Current Protocols in Molecular Biology Vol. I*, 8.3.1-8.3.6, F. Ausubel *et al.*, eds, J. Wiley and Sons, New York).

In another general approach, the addition of amino acids to a peptide or peptides found to be active, can be carried out using various methods. In one, the sequences of peptides selected in early panning are determined individually and new oligonucleotides, incorporating the determined sequence and an adjoining degenerate sequence, are synthesized. These are then cloned to produce a secondary library. Alternatively, methods can be used to add a second IR or IGF-1R binding sequence to a pool of peptide-bearing phage. In accordance with one method, a restriction site is installed next to the first IR or IGF-1R binding sequence. Preferably, the enzyme should cut outside of its recognition sequence. The recognition site may be placed several bases from the first binding sequence. To insert a second IR or IGF-1R binding sequence, the pool of phage DNA is digested

and blunt-ended by filling in the overhang with Klenow fragment. Double-stranded, blunt-ended, degenerately synthesized oligonucleotides are then ligated into this site to produce a second binding sequence juxtaposed to the first binding sequence. This secondary library is then amplified and screened as before.

While in some instances it may be appropriate to synthesize longer peptides to bind certain receptors, in other cases it may be desirable to provide peptides having two or more IR or IGF-1R binding sequences separated by spacer (e.g., linker) residues. For example, the binding sequences may be separated by spacers that allow the regions of the peptides to be presented to the receptor in different ways. The distance between binding regions may be as little as 1 residue, or at least 2-20 residues, or up to at least 100 residues. Preferred spacers are 3, 6, 9, 12, 15, or 18 residues in length. For probing large binding sites or tandem binding sites (e.g., Site 1 and Site 2 of IR), the binding regions may be separated by a spacer of residues of up to 20 to 30 amino acids. The number of spacer residues when present will typically be at least 2 residues, and often will be less than 20 residues.

The oligonucleotide library may have binding sequences which are separated by spacers (e.g., linkers), and thus may be represented by the formula: $(NNK)y - (abc)_n - (NNK)_z$ where N and K are as defined previously (note that S as defined previously may be substituted for K), and y+z is equal to about 5, 6, 7, 8, or more, a, b and c represent the same or different nucleotides comprising a codon encoding spacer amino acids, n is up to about 3, 6, 9, or 12 amino acids, or more. The spacer residues may be somewhat flexible, comprising oligo-glycine, or oligo-glycine-glycine-serine, for example, to provide the diversity domains of the library with the ability to interact with sites in a large binding site relatively unconstrained by attachment to the phage protein. Rigid spacers, such as, e.g., oligo-proline, may also be inserted separately or in combination with other spacers, including glycine spacers. It may be desired to have the IR or IGF-1R

binding sequences close to one another and use a spacer to orient the binding sequences with respect to each other, such as by employing a turn between the two sequences, as might be provided by a spacer of the sequence glycine-proline-glycine, for example. To add stability to such a turn, it may be desirable or necessary to add cysteine residues at either or both ends of each variable region. The cysteine residues would then form disulfide bridges to hold the variable regions together in a loop, and in this fashion may also serve to mimic a cyclic peptide. Of course, those skilled in the art will appreciate that various other types of covalent linkages for cyclization may also be used.

Spacer residues as described above may also be situated on either or both ends of the IR or IGF-1R binding sequences. For instance, a cyclic peptide may be designed without an intervening spacer, by having a cysteine residue on both ends of the peptide. As described above, flexible spacers, e.g., oligo-glycine, may facilitate interaction of the peptide with the selected receptors. Alternatively, rigid spacers may allow the peptide to be presented as if on the end of a rigid arm, where the number of residues, e.g., proline residues, determines not only the length of the arm but also the direction for the arm in which the peptide is oriented. Hydrophilic spacers, made up of charged and/or uncharged hydrophilic amino acids, (e.g., Thr, His, Asn, Gln, Arg, Glu, Asp, Met, Lys, etc.), or hydrophobic spacers of hydrophobic amino acids (e.g., Phe, Leu, Ile, Gly, Val, Ala, etc.) may be used to present the peptides to receptor binding sites with a variety of local environments.

Notably, some peptides, because of their size and/or sequence, may cause severe defects in the infectivity of their carrier phage. This causes a loss of phage from the population during reinfection and amplification following each cycle of panning. To minimize problems associated with defective infectivity, DNA prepared from the eluted phage can be transformed into appropriate host cells, such as, e.g., *E. coli*, preferably by electroporation (see, e.g., Dower *et al.*, *Nucl. Acids Res.* **16**:6127-6145), or

well-known chemical means. The cells are cultivated for a period of time sufficient for marker expression, and selection is applied as typically done for DNA transformation. The colonies are amplified, and phage harvested for affinity enrichment in accordance with established methods. Phage identified in the affinity enrichment may be re-amplified by infection into the host cells. The successful transformants are selected by growth in an appropriate antibiotic(s), e.g., tetracycline or ampicillin. This may be done on solid or in liquid growth medium.

For growth on solid medium, the cells are grown at a high density (about 10^8 to 10^9 transformants per m^2) on a large surface of, for example, L-agar containing the selective antibiotic to form essentially a confluent lawn. The cells and extruded phage are scraped from the surface and phage are prepared for the first round of panning (see, e.g., Parmley and Smith, 1988, *Gene* **73**:305-318). For growth in liquid culture, cells may be grown in L-broth and antibiotic through about 10 or more doublings. The phage are harvested by standard procedures (see Sambrook *et al.*, 1989, *Molecular Cloning*, 2nd ed.). Growth in liquid culture may be more convenient because of the size of the libraries, while growth on solid media likely provides less chance of bias during the amplification process.

For affinity enrichment of desired clones, generally about 10^3 to 10^4 library equivalents (a library equivalent is one of each recombinant; 10^4 equivalents of a library of 10^9 members is $10^9 \times 10^4 = 10^{13}$ phage), but typically at least 10^2 library equivalents, up to about 10^5 to 10^6, are incubated with a receptor (or portion thereof) to which the desired peptide is sought. The receptor is in one of several forms appropriate for affinity enrichment schemes. In one example the receptor is immobilized on a surface or particle, and the library of phage bearing peptides is then panned on the immobilized receptor generally according to procedures known in the art. In an alternate scheme, a receptor is attached to a recognizable ligand (which may be attached via a tether). A specific example of such a ligand is biotin. The receptor, so modified, is incubated with the library of phage and

binding occurs with both reactants in solution. The resulting complexes are then bound to streptavidin (or avidin) through the biotin moiety. The streptavidin may be immobilized on a surface such as a plastic plate or on particles, in which case the complexes (phage/peptide/receptor/biotin/streptavidin) are physically retained; or the streptavidin may be labeled, with a fluorophor, for example, to tag the active phage/peptide for detection and/or isolation by sorting procedures, e.g., on a fluorescence-activated cell sorter.

Phage that associate with IR or IGF-1R via non-specific interactions are removed by washing. The degree and stringency of washing required will be determined for each receptor/peptide of interest. A certain degree of control can be exerted over the binding characteristics of the peptides recovered by adjusting the conditions of the binding incubation and the subsequent washing. The temperature, pH, ionic strength, divalent cation concentration, and the volume and duration of the washing will select for peptides within particular ranges of affinity for the receptor. Selection based on slow dissociation rate, which is usually predictive of high affinity, is the most practical route. This may be done either by continued incubation in the presence of a saturating amount of free ligand, or by increasing the volume, number, and length of the washes. In each case, the rebinding of dissociated peptide-phage is prevented, and with increasing time, peptide-phage of higher and higher affinity are recovered. Additional modifications of the binding and washing procedures may be applied to find peptides that bind receptors under special conditions. Once a peptide sequence that imparts some affinity and specificity for the receptor molecule is known, the diversity around this binding motif may be embellished. For instance, variable peptide regions may be placed on one or both ends of the identified sequence. The known sequence may be identified from the literature, or may be derived from early rounds of panning in the context of the present invention.

G. Screening Assays

In another embodiment of this invention, screening assays to identify pharmacologically active ligands at IR and/or IGF-1R are provided. Ligands may encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 daltons. Such ligands can comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. Ligands often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Ligands can also comprise biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs, or combinations thereof.

Ligands may include, for example, 1) peptides such as soluble peptides, including Ig-tailed fusion peptides and members of random peptide libraries (see, e.g., Lam *et al.*, 1991, *Nature* **354**:82-84; Houghten *et al.*, 1991, *Nature* **354**:84-86) and combinatorial chemistry-derived molecular libraries made of D- and/or L-configuration amino acids; 2) phosphopeptides (e.g., members of random and partially degenerate, directed phosphopeptide libraries, see, e.g., Songyang *et al.*, 1993, *Cell* **72**:767-778); 3) antibodies (e.g., polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, and single chain antibodies as well as Fab, $F(ab')_2$, Fab expression library fragments, and epitope-binding fragments of antibodies); and 4) small organic and inorganic molecules.

Ligands can be obtained from a wide variety of sources including libraries of synthetic or natural compounds. Synthetic compound libraries are commercially available from, for example, Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, NJ), Brandon Associates

(Merrimack, NH), and Microsource (New Milford, CT). A rare chemical library is available from Aldrich Chemical Company, Inc. (Milwaukee, WI). Natural compound libraries comprising bacterial, fungal, plant or animal extracts are available from, for example, Pan Laboratories (Bothell, WA). In addition, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides.

Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts can be readily produced. Methods for the synthesis of molecular libraries are readily available (see, e.g., DeWitt *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* **90**:6909; Erb *et al.*, 1994, *Proc. Natl. Acad. Sci. USA* **91**:11422; Zuckermann *et al.*, 1994, *J. Med. Chem.* **37**:2678; Cho *et al.*, 1993, *Science* **261**:1303; Carell *et al.*, 1994, *Angew. Chem. Int. Ed. Engl.* **33**:2059; Carell *et al.*, 1994, *Angew. Chem. Int. Ed. Engl.* **33**:2061; and in Gallop *et al.*, 1994, *J. Med. Chem.* **37**:1233). In addition, natural or synthetic compound libraries and compounds can be readily modified through conventional chemical, physical and biochemical means (see, e.g., Blondelle *et al.*, 1996, *Trends in Biotech.* **14**:60), and may be used to produce combinatorial libraries. In another approach, previously identified pharmacological agents can be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, and the analogs can be screened for IR-modulating activity.

Numerous methods for producing combinatorial libraries are known in the art, including those involving biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide or peptide libraries, while the other four approaches are applicable to polypeptide, peptide, non-peptide oligomer, or small molecule libraries of compounds (K. S. Lam, 1997, *Anticancer Drug Des.* **12**:145).

Libraries may be screened in solution by methods generally known in the art for determining whether ligands competitively bind at a common binding site. Such methods may including screening libraries in solution (e.g., Houghten, 1992, *Biotechniques* **13**:412-421), or on beads (Lam, 1991, *Nature* **354**:82-84), chips (Fodor, 1993, *Nature* **364**:555-556), bacteria or spores (Ladner U.S. Pat. No. 5,223,409), plasmids (Cull *et al.*, 1992, *Proc. Natl. Acad. Sci. USA* **89**:1865-1869), or on phage (Scott and Smith, 1990, *Science* **249**:386-390; Devlin, 1990, *Science* **249**:404-406; Cwirla *et al.*, 1990, *Proc. Natl. Acad. Sci. USA* **97**:6378-6382; Felici, 1991, *J. Mol. Biol.* **222**:301-310; Ladner, *supra*).

Where the screening assay is a binding assay, IR, or one of the IR-binding peptides disclosed herein, may be joined to a label, where the label can directly or indirectly provide a detectable signal. Various labels include radioisotopes, fluorescent molecules, chemiluminescent molecules, enzymes, specific binding molecules, particles, e.g., magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin, etc. For the specific binding members, the complementary member would normally be labeled with a molecule that provides for detection, in accordance with known procedures.

A variety of other reagents may be included in the screening assay. These include reagents like salts, neutral proteins, e.g., albumin, detergents, etc., which are used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used. The components are added in any order that produces the requisite binding. Incubations are performed at any temperature that facilitates optimal activity, typically between 4° and 40°C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Normally, between 0.1 and 1 h will be sufficient. In general, a plurality of assay mixtures is run in parallel with different test agent concentrations to obtain a differential

response to these concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration or below the level of detection.

The screening assays provided in accordance with this invention are based on those disclosed in International application WO 96/04557, which is incorporated herein in its entirety. Briefly, WO 96/04557 discloses the use of reporter peptides that bind to active sites on targets and possess agonist or antagonist activity at the target. These reporters are identified from recombinant libraries and are either peptides with random amino acid sequences or variable antibody regions with at least one CDR region that has been randomized (rVab). The reporter peptides may be expressed in cell recombinant expression systems, such as for example in *E. coli*, or by phage display (see WO 96/04557 and Kay *et al.* 1996, *Mol. Divers.* **1**(2):139-40, both of which are incorporated herein by reference). The reporters identified from the libraries may then be used in accordance with this invention either as therapeutics themselves, or in competition binding assays to screen for other molecules, preferably small, active molecules, which possess similar properties to the reporters and may be developed as drug candidates to provide agonist or antagonist activity. Preferably, these small organic molecules are orally active.

The basic format of an *in vitro* competitive receptor binding assay as the basis of a heterogeneous screen for small organic molecular replacements for insulin may be as follows: occupation of the active site of IR is quantified by time-resolved fluorometric detection (TRFD) with streptavidin-labeled europium (saEu) complexed to biotinylated peptides (bP). In this assay, saEu forms a ternary complex with bP and IR (i.e., IR:bP:saEu complex). The TRFD assay format is well-established, sensitive, and quantitative (Tompkins *et al.,* 1993, *J. Immunol. Methods* **163**:209-216). The assay can use a single-chain antibody or a biotinylated peptide. Furthermore, both assay formats faithfully report the competition of the biotinylated ligands binding to the active site of IR by insulin.

In these assays, soluble IR is coated on the surface of microtiter wells, blocked by a solution of 0.5% bovine serum albumin (BSA) and 2% non-fat milk in PBS, and then incubated with biotinylated peptide or rVab. Unbound bP is then washed away and saEu is added to complex with receptor-bound bP. Upon addition of the acidic enhancement solution, the bound europium is released as free Eu^{3+} which rapidly forms a highly fluorescent and stable complex with components of the enhancement solution. The IR:bP bound saEu is then converted into its highly fluorescent state and detected by a detector such as Wallac Victor II (EG&G Wallac, Inc.)

Phage display libraries can also be screened for ligands that bind to IR or IGF-1R, as described above. Details of the construction and analyses of these libraries, as well as the basic procedures for biopanning and selection of binders, have been published (see, e.g., WO 96/04557; Mandecki *et al.*, 1997, *Display Technologies – Novel Targets and Strategies*, P. Guttry (ed), International Business Communications, Inc. Southborogh, MA, pp. 231-254; Ravera *et al.*, 1998, *Oncogene* **16**:1993-1999; Scott and Smith, 1990, *Science* **249**:386-390); Grihalde *et al.*, 1995, *Gene* **166**:187-195; Chen *et al.*, 1996, *Proc. Natl. Acad. Sci. USA* **93**:1997-2001; Kay *et al.*, 1993, *Gene* **128**:59-65; Carcamo *et al.*, 1998, *Proc. Natl. Acad. Sci. USA* **95**:11146-11151; Hoogenboom, 1997, *Trends Biotechnol.* **15**:62-70; Rader and Barbas, 1997, *Curr. Opin. Biotechnol.* **8**:503-508; all of which are incorporated herein by reference).

The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g., peptides are generally unsuitable active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis, and testing

are generally used to avoid large-scale screening of molecules for a target property.

There are several steps commonly taken in the design of a mimetic from a compound having a given target property. First, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide (e.g., by substituting each residue in turn). These parts or residues constituting the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modeled according to its physical properties (e.g., stereochemistry, bonding, size, and/or charge), using data from a range of sources (e.g., spectroscopic techniques, X-ray diffraction data, and NMR). Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms), and other techniques can be used in this modeling process.

In a variant of this approach, the three dimensional structure of the ligand and its binding partner are modeled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this in the design of the mimetic.

A template molecule is then selected, and chemical groups that mimic the pharmacophore can be grafted onto the template. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the mimetic is easy to synthesize, is likely to be pharmacologically acceptable, does not degrade *in vivo*, and retains the biological activity of the lead compound. The mimetics found are then screened to ascertain the extent they exhibit the target property, or to what extent they inhibit it. Further optimization or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

This invention provides specific IR and IGF-1R amino acid sequences that function as either agonists or antagonists at IR and/or IGF-1R.

Additional sequences may be obtained in accordance with the procedures described herein.

H. Use of the Peptides Provided by this Invention

The IR and IGF-1R agonist and antagonist peptides provided by this invention are useful as lead compounds for identifying other more potent or selective therapeutics, assay reagents for identifying other useful ligands by, for example, competition screening assays, as research tools for further analysis of IR and IGF-1R, and as potential therapeutics in pharmaceutical compositions. In one embodiment, one or more of the disclosed peptides can be provided as components in a kit for identifying other ligands (e.g., small, organic molecules) that bind to IR or IGF-1R. Such kits may also comprise IR or IGF-1R, or functional fragments thereof. The peptide and receptor components of the kit may be labeled (e.g., by radioisotopes, fluorescent molecules, chemiluminescent molecules, enzymes or other labels), or may be unlabeled and labeling reagents may be provided. The kits may also contain peripheral reagents such as buffers, stabilizers, etc. Instructions for use can also be provided.

In another embodiment, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which are derived from the peptide sequences, and include members that bind to Site 1 and/or Site 2 of IR or IGF-1R. Such libraries can be used to identify sequence variants that increase or otherwise modulate the binding and/or activity of the original peptide at IR or IGF-1R, as described in the related applications of Beasley *et al.* International Application PCT/US00/08528, filed March 29, 2000, and Beasley *et al.*, U.S. Application Serial No. 09/538,038, filed March 29, 2000, in accordance with well-established techniques.

IR agonist amino acid sequences provided by this invention are useful as insulin analogs and may therefore be developed as treatments for diabetes or other diseases associated with a decreased response or

production of insulin. For use as an insulin supplement or replacement, amino acid sequences include D117/H2C: FHENFYDWFVRQVSK (SEQ ID NO:1780); D117/H2C minus terminal lysine: FHENFYDWFVRQVS (SEQ ID NO:1557); D118: DYKDFYDAIQLVRSARAGGTRDKK (SEQ ID NO:1781); D118 minus FLAG® tag and terminal lysines: FYDAIQLVRSARAGGTRD (SEQ ID NO:1782); D119: KDRAFYNGLRDLVGAVYGAWDKK (SEQ ID NO:1733); D119 minus terminal lysines: KDRAFYNGLRDLVGAVYGAWD (residues 1-21 of SEQ ID NO:1733); D116/JBA5: DYKDLCQSWGVRIGWLAGLCPKK (SEQ ID NO:1541); D116/JBA5 minus FLAG® tag and terminal lysines: LCQSWGVRIGWLAGLCP (SEQ ID NO:1542); D113/H2: DYKDVTFTSAVFHENFYDWFVRQVSKK (SEQ ID NO:1783); D113/H2 minus FLAG® tag and terminal lysines: VTFTSAVFHENFYDWFVRQVS (SEQ ID NO:1784); and S175: GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560). Preferred peptide dimer sequences are represented by S325, S332, S333, S335, S337, S353, S374-S376, S378, S379, S381, S414, S415, and S418 (see Table 7). Other preferred dimers sequences are represented by S455, S457, S458, S467, S468, S471, S499, S510, S518, S519, and S520 sequences (see Table 7). Especially preferred is the S519 dimer sequence, which shows *in vitro* and *in vivo* activity comparable to insulin (see Figures 31A-C, 32A-B, and 33).

IGF-1R antagonist amino acid sequences provided by this invention are useful as treatments for cancers, including, but not limited to, breast, prostate, colorectal, and ovarian cancers. Human and breast cancers are responsible for over 40,000 deaths per year, as present treatments such as surgery, chemotherapy, radiation therapy, and immunotherapy show limited success. The IGF-1R antagonist amino acid sequences disclosed herein are also useful for the treatment or prevention of diabetic retinopathy. Recent reports have shown that a previously identified IGF-1R antagonist can suppress retinal neovascularization, which causes diabetic retinopathy (Smith *et al.*, 1999, *Nat. Med.* **5**:1390-1395). Preferred IGF-1R antagonist

amino acid sequences include those comprising the sequences of RP33-IGF and RP33K-IGF (Tables 24-26).

IGF-1R agonist amino acid sequences provided by this invention are useful for development as treatments for neurological disorders, including stroke and diabetic neuropathy. Reports of several different groups implicate IGF-1R in the reduction of global brain ischemia, and support the use of IGF-1 for the treatment of diabetic neuropathy (reviewed in Auer *et al.*, 1998, *Neurology* **51**:S39-S43; Apfel, 1999, *Am. J. Med.* **107**:34S-42S). The IGF-1R agonist peptides of the invention may be useful for enhancing the survival of cells and/or blocking apoptosis in cells. Preferred IGF-1R agonist amino acid sequences include those comprising the sequences of G33, RP48, RP60, and RP30-IGF-12-RP31-IGF (Tables 27-29).

I. Methods of Administration

The amino acid sequences of this invention may be administered as pharmaceutical compositions comprising standard carriers known in the art for delivering proteins and peptides and by gene therapy. Preferably, a pharmaceutical composition includes, in admixture, a pharmaceutically (i.e., physiologically) acceptable carrier, excipient, or diluent, and one or more of an IR or IGF-1R agonist or antagonist peptide, as an active ingredient. The preparation of pharmaceutical compositions that contain peptides as active ingredients is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients that are pharmaceutically (i.e., physiologically) acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary

substances such as wetting or emulsifying agents, pH-buffering agents, which enhance the effectiveness of the active ingredient.

An IR or IGF-1R agonist or antagonist peptide can be formulated into a pharmaceutical composition as neutralized physiologically acceptable salt forms. Suitable salts include the acid addition salts (i.e., formed with the free amino groups of the peptide molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The pharmaceutical compositions can be administered systemically by oral or parenteral routes. Non-limiting parenteral routes of administration include subcutaneous, intramuscular, intraperitoneal, intravenous, transdermal, inhalation, intranasal, intra-arterial, intrathecal, enteral, sublingual, or rectal. Due to the labile nature of the amino acid sequences parenteral administration is preferred. Preferred modes of administration include aerosols for nasal or bronchial absorption; suspensions for intravenous, intramuscular, intrasternal or subcutaneous, injection; and compounds for oral administration.

Intravenous administration, for example, can be performed by injection of a unit dose. The term "unit dose" when used in reference to a pharmaceutical composition of the present invention refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., liquid used to dilute a concentrated or pure substance (either liquid or solid), making that substance the correct (diluted) concentration for use. For injectable administration, the composition is in sterile solution or suspension or may be emulsified in pharmaceutically- and physiologically-acceptable aqueous or

oleaginous vehicles, which may contain preservatives, stabilizers, and material for rendering the solution or suspension isotonic with body fluids (i.e., blood) of the recipient.

Excipients suitable for use are water, phosphate buffered saline, pH 7.4, 0.15 M aqueous sodium chloride solution, dextrose, glycerol, dilute ethanol, and the like, and mixtures thereof. Illustrative stabilizers are polyethylene glycol, proteins, saccharides, amino acids, inorganic acids, and organic acids, which may be used either on their own or as admixtures. The amounts or quantities, as well as routes of administration, used are determined on an individual basis, and correspond to the amounts used in similar types of applications or indications known to those of skill in the art.

Pharmaceutical compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the subject's immune system to utilize the active ingredient, and degree of modulation of IR or IGF-1R activity desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are specific for each individual. However, suitable dosages may range from about 10 to 200 nmol active peptide per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusions sufficient to maintain picomolar concentrations (e.g., approximately 1 pM to approximately 10 nM) in the blood are contemplated. An exemplary formulation comprises the IR or IGF-1R agonist or antagonist peptide in a mixture with sodium busulfite USP (3.2 mg/ml); disodium edetate USP (0.1 mg/ml); and water for injection q.s.a.d. (1 ml).

Further guidance in preparing pharmaceutical formulations can be found in, e.g., Gilman *et al.* (eds), 1990, *Goodman and Gilman's: The*

Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press; and *Remington's Pharmaceutical Sciences*, 17th ed., 1990, Mack Publishing Co., Easton, PA; Avis *et al.* (eds), 1993, *Pharmaceutical Dosage Forms: Parenteral Medications*, Dekker, New York; Lieberman *et al.* (eds), 1990, *Pharmaceutical Dosage Forms: Disperse Systems*, Dekker, New York.

The present invention further contemplates compositions comprising an IR or IGF-1R agonist or antagonist peptide, and a physiologically acceptable carrier, excipient, or diluent as described in detail herein.

The constructs as described herein may also be used in gene transfer and gene therapy methods to allow the expression of one or more amino acid sequences of the present invention. The amino acid sequences of the present invention can be used for gene therapy and thereby provide an alternative method of treating diabetes which does not rely on the administration or expression of insulin. Expressing insulin for use in gene therapy requires the expression of a precursor product, which must then undergo processing including cleavage and disulfide bond formation to form the active product. The amino acid sequences of this invention, which possess activity, are relatively small, and thus do not require the complex processing steps to become active. Accordingly, these sequences provide a more suitable product for gene therapy.

Gene transfer systems known in the art may be useful in the practice of the gene therapy methods of the present invention. These include viral and non-viral transfer methods. A number of viruses have been used as gene transfer vectors, including polyoma, *i.e.*, SV40 (Madzak *et al.*, 1992, *J. Gen. Virol.*, **73**:1533-1536), adenovirus (Berkner, 1992, *Curr. Top. Microbiol. Immunol.*, **158**:39-6; Berkner *et al.*, 1988, *Bio Techniques*, **6**:616-629; Gorziglia *et al.*, 1992, *J. Virol.*, **66**:4407-4412; Quantin *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, **89**:2581-2584; Rosenfeld *et al.*, 1992, *Cell*, **68**:143-155; Wilkinson *et al.*, 1992, *Nucl. Acids Res.*, **20**:2233-2239; Stratford-Perricaudet *et al.*, 1990, *Hum. Gene Ther.*, **1**:241-256), vaccinia virus (Mackett *et al.*, 1992, *Biotechnology*, **24**:495- 499), adeno-associated virus

(Muzyczka, 1992, *Curr. Top. Microbiol. Immunol.* **158**:91- 123; Ohi *et al.*, 1990, *Gene*, **89**:279-282), herpes viruses including HSV and EBV (Margolskee, 1992, *Curr. Top. Microbiol. Immunol.* **158**:67-90; Johnson *et al.*, 1992, *J. Virol.*, **66**:2952-2965; Fink *et al.*, 1992, *Hum. Gene Ther.* **3**:11-19; Breakfield *et al.*, 1987, *Mol. Neurobiol.*, **1**:337-371; Fresse *et al.*, 1990, *Biochem. Pharmacol.* **40**:2189-2199), and retroviruses of avian (Brandyopadhyay *et al.*, 1984, *Mol. Cell Biol.*, **4**:749-754; Petropouplos *et al.*, 1992, *J. Virol.*, **66**:3391-3397), murine (Miller, 1992, *Curr. Top. Microbiol. Immunol.* **158**:1-24; Miller *et al.*, 1985, *Mol. Cell Biol.*, **5**:431-437; Sorge *et al.*, 1984, *Mol. Cell Biol.*, **4**:1730-1737; Mann *et al.*, 1985, *J. Virol.*, **54**:401-407), and human origin (Page *et al.*, 1990, *J. Virol.*, **64**:5370-5276; Buchschalcher *et al.*, 1992, *J. Virol.*, **66**:2731-2739). Most human gene therapy protocols have been based on disabled murine retroviruses.

Non-viral gene transfer methods known in the art include chemical techniques such as calcium phosphate coprecipitation (Graham *et al.*, 1973, *Virology*, **52**:456-467; Pellicer *et al.*, 1980, *Science*, **209**:1414-1422), mechanical techniques, for example microinjection (Anderson *et al.*, 1980, *Proc. Natl. Acad. Sci. USA*, **77**:5399-5403; Gordon *et al.*, 1980, *Proc. Natl. Acad. Sci. USA*, **77**:7380-7384; Brinster *et al.*, 1981, *Cell*, **27**:223-231; Constantini *et al.*, 1981, *Nature*, **294**:92-94), membrane fusion-mediated transfer via liposomes (Felgner *et al.*, 1987, *Proc. Natl. Acad. Sci. USA*, **84**:7413-7417; Wang *et al.*, 1989, *Biochemistry*, **28**:9508-9514; Kaneda *et al.*, 1989, *J. Biol. Chem.*, **264**:12126-12129; Stewart *et al.*, 1992, *Hum. Gene Ther.* **3**:267-275; Nabel *et al.*, 1990, *Science*, **249**:1285-1288; Lim *et al.*, 1992, *Circulation*, **83**:2007-2011; U.S. Patent Nos. 5,283,185 and 5,795,587), and direct DNA uptake and receptor-mediated DNA transfer (Wolff *et al.*, 1990, *Science*, **247**:1465-1468; Wu *et al.*, 1991, *BioTechniques*, **11**:474-485; Zenke *et al.*, 1990, *Proc. Natl. Acad. Sci. USA*, **87**:3655-3659; Wu *et al.*, 1989, *J. Biol. Chem.*, **264**:16985-16987; Wolff *et al.*, 1991, *BioTechniques*, **11**:474-485; Wagner *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, **88**:4255-4259; Cotten *et al.*, 1990, *Proc. Natl. Acad. Sci.*

USA, **87**:4033-4037; Curiel *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, **88**:8850-8854; Curiel *et al.*, 1991, *Hum. Gene Ther.* **3**:147-154).

Many types of cells and cell lines (e.g., primary cell lines or established cell lines) and tissues are capable of being stably transfected by or receiving the constructs of the invention. Examples of cells that may be used include, but are not limited to, stem cells, B lymphocytes, T lymphocytes, macrophages, other white blood lymphocytes (e.g., myelocytes, macrophages, or monocytes), immune system cells of different developmental stages, erythroid lineage cells, pancreatic cells, lung cells, muscle cells, liver cells, fat cells, neuronal cells, glial cells, other brain cells, transformed cells of various cell lineages corresponding to normal cell counterparts (e.g., K562, HEL, HL60, and MEL cells), and established or otherwise transformed cells lines derived from all of the foregoing. In addition, the constructs of the present invention may be transferred by various means directly into tissues, where they would stably integrate into the cells comprising the tissues. Further, the constructs containing the DNA sequences of the peptides of the invention can be introduced into primary cells at various stages of development, including the embryonic and fetal stages, so as to effect gene therapy at early stages of development.

In one approach, plasmid DNA is complexed with a polylysine-conjugated antibody specific to the adenovirus hexon protein, and the resulting complex is bound to an adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector permits efficient binding, internalization, and degradation of the endosome before the coupled DNA is damaged.

In another approach, liposome/DNA is used to mediate direct *in vivo* gene transfer. While in standard liposome preparations the gene transfer process is non-specific, localized *in vivo* uptake and expression have been reported in tumor deposits, for example, following direct *in situ* administration (Nabel, 1992, *Hum. Gene Ther.* **3**:399-410).

Suitable gene transfer vectors possess a promoter sequence, preferably a promoter that is cell-specific and placed upstream of the sequence to be expressed. The vectors may also contain, optionally, one or more expressible marker genes for expression as an indication of successful transfection and expression of the nucleic acid sequences contained in the vector. In addition, vectors can be optimized to minimize undesired immunogenicity and maximize long-term expression of the desired gene product(s) (see Nabe, 1999, *Proc. Natl. Acad. Sci. USA* **96**:324-326). Moreover, vectors can be chosen based on cell-type that is targeted for treatment.

Illustrative examples of vehicles or vector constructs for transfection or infection of the host cells include replication-defective viral vectors, DNA virus or RNA virus (retrovirus) vectors, such as adenovirus, herpes simplex virus and adeno-associated viral vectors. Adeno-associated virus vectors are single stranded and allow the efficient delivery of multiple copies of nucleic acid to the cell's nucleus. Preferred are adenovirus vectors. The vectors will normally be substantially free of any prokaryotic DNA and may comprise a number of different functional nucleic acid sequences. An example of such functional sequences may be a DNA region comprising transcriptional and translational initiation and termination regulatory sequences, including promoters (e.g., strong promoters, inducible promoters, and the like) and enhancers which are active in the host cells. Also included as part of the functional sequences is an open reading frame (polynucleotide sequence) encoding a protein of interest. Flanking sequences may also be included for site-directed integration. In some situations, the 5'-flanking sequence will allow homologous recombination, thus changing the nature of the transcriptional initiation region, so as to provide for inducible or non-inducible transcription to increase or decrease the level of transcription, as an example.

In general, the encoded and expressed peptide may be intracellular, i.e., retained in the cytoplasm, nucleus, or in an organelle, or may be

secreted by the cell. For secretion, a signal sequence may be fused to the peptide sequence. As previously mentioned, a marker may be present for selection of cells containing the vector construct. The marker may be an inducible or non-inducible gene and will generally allow for positive selection under induction, or without induction, respectively. Examples of marker genes include neomycin, dihydrofolate reductase, glutamine synthetase, and the like. The vector employed will generally also include an origin of replication and other genes that are necessary for replication in the host cells, as routinely employed by those having skill in the art. As an example, the replication system comprising the origin of replication and any proteins associated with replication encoded by a particular virus may be included as part of the construct. The replication system must be selected so that the genes encoding products necessary for replication do not ultimately transform the cells. Such replication systems are represented by replication-defective adenovirus (see G. Acsadi *et al.*, 1994, H*um. Mol. Genet.* **3**:579-584) and by Epstein-Barr virus. Examples of replication defective vectors, particularly, retroviral vectors that are replication defective, are BAG, (see Price *et al.*, 1987, *Proc. Natl. Acad. Sci. USA*, **84**:156; Sanes *et al.*, 1986, *EMBO J.*, **5**:3133). It will be understood that the final gene construct may contain one or more genes of interest, for example, a gene encoding a bioactive metabolic molecule. In addition, cDNA, synthetically produced DNA or chromosomal DNA may be employed utilizing methods and protocols known and practiced by those having skill in the art.

According to one approach for gene therapy, a vector encoding an IR or IGF-1R agonist or antagonist peptide is directly injected into the recipient cells (*in vivo* gene therapy). Alternatively, cells from the intended recipients are explanted, genetically modified to encode an IR or IGF-1R agonist or antagonist peptide, and reimplanted into the donor (*ex vivo* gene therapy). An *ex vivo* approach provides the advantage of efficient viral gene transfer, which is superior to in vivo gene transfer approaches. In accordance with *ex*

vivo gene therapy, the host cells are first transfected with engineered vectors containing at least one gene encoding an IR or IGF-1R agonist or antagonist peptide, suspended in a physiologically acceptable carrier or excipient such as saline or phosphate buffered saline, and the like, and then administered to the host or host cells. The desired gene product is expressed by the injected cells, which thus introduce the gene product into the host. The introduced gene products can thereby be utilized to treat or ameliorate a disorder that is related to altered insulin or IGF-1 levels (e.g., diabetes).

The described constructs may be administered in the form of a pharmaceutical preparation or composition containing a pharmaceutically acceptable carrier and a physiological excipient, in which preparation the vector may be a viral vector construct, or the like, to target the cells, tissues, or organs of the recipient organism of interest, including human and non-human mammals. The composition may be formed by dispersing the components in a suitable pharmaceutically acceptable liquid or solution such as sterile physiological saline or other injectable aqueous liquids. The amounts of the components to be used in such compositions may be routinely determined by those having skill in the art. The compositions may be administered by parenteral routes of injection, including subcutaneous, intravenous, intramuscular, and intrasternal.

J. Cancer Therapeutics

In recent experiments, embryo fibroblasts from IGF-1R knock-out mice have been shown to be highly resistant to transformation by oncogenes such as SV40 T antigen, activated Ha-ras, activated Src, and others (B. Valentinis and R. Baserga, 2001, *Mol. Pathol.*, **54**:133-137). This suggested that IGF-1R was required to mediate malignant transformation by these oncogenes. In addition, IGF-1 and IGF-1R have been shown to act as transforming factors in various forms of human cancer (see above). IGF-1 and IGF-2 have also been implicated as factors in the malignant

transformation of several tissues. Transgenic mice that express a truncated form of IGF-1 that has a decreased affinity for IGFBPs (des(1-3) IGF-1I), show increased incidence of mammary tumors (Hadsell *et al.*, 2000, *Oncogene* **19**:889-898). In addition, mice over-expressing IGF-1I in mammary glands showed increased mammary tumor formation (Bates *et al.*, 1995, *Br. J. Cancer* **72**:1189-1193). Transgenic mice that overexpress IGF-1 in the basal layer of the skin show hyperplasia of the epidermis and increased promotion of spontaneous tumors (DiGiovanni *et al.*, 2000, *Cancer Res.* **60**:1561-1570).

IGF-1R also appears to cross-talk with other hormone receptors. Considerable evidence suggests that estrogen can act to increase expression of IGF-1R. This is of particular importance in breast cancer, where the expression of IGF-1R correlates with expression of the estrogen receptor (ER). IGF-1R expression is higher in tumors from ER positive patients. Accordingly, IGF-1R expression could be used as a prognostic marker for breast cancer patients. In addition, high levels of IRS-1, a key intermediate in the IGF-1R signal transduction cascade, correlates with tumor size and shorter disease-free survival in patients with ER positive tumors (D. Sachdev and D. Yee, 2001, *Endocr. Relat. Cancer* **8**:197-209). In addition, treatment with anti-estrogens has been shown to decrease the expression of IGF-1R and IRS-1 (Chan *et al.*, 2001, *Clin. Cancer Res.* **7**:2545-2554). Thus, the cross-talk between IGF-1R and ER may be complex. Yet, it is clear that IGF-signaling promotes malignant transformation in mammary glands. Interestingly, ER positive MCF-7 cells treated with IGF-1 show a sustained activation of the PI3K-Akt pathway and protection against apoptosis induced by serum deprivation. In contrast, ER negative MDA-MB 231 cells show only a transient activation of PI3K-Akt pathway (Bartucci *et al.*, 2001, *Cancer Res.* **61**:6747-6754).

Studies have also revealed a connection between IGF-1R-mediated signaling and epidermal growth factor (EGF)-induced signaling through ErbB-receptors. IGF-1R and ErbB-2 (Neu/Her2) have been observed to

form hetero-oligomers induced by stimulation with heregulin or IGF-1 (Balana *et al.*, 2001, *Oncogene*, **19**:34-47, 2001). In glioblastomas, resistance to a chemical inhibitor of the ErbB receptor tyrosine kinase has been correlated with increased IGF-1R expression and constitutive PI3K signaling (Chakravarti *et al.*, 2002, *Cancer Res.* **62**:200-207). In breast cancer cell lines over expressing ErbB-2, increased IGF-1R signaling was observed in the presence of the anti-ErbB-2 receptor monoclonal antibody Herceptin®/trastuzumab (Lu *et al.*, 2001, *J. Natl. Cancer Inst.* **93**:1852-1857).

Modulation of IGF-signaling in various malignant cells has provided further evidence for the involvement of the IGF-1R in cancer. Abrogation of IGF-1R expression by antisense RNA reversed the transformed phenotype in cervical cancer cells. Antisense to IGF-1R also inhibited glioblastoma and melanoma xenografts in nude mice (Resnicoff *et al.*, 1994, *Cancer Res.* **54**:4848-4850; Resnicoff *et al.*, 1994, *Cancer Res.* **54**:2218-2222; Nakamura *et al.*, 2000, *Cancer Res.* **60**:760-765, 2000). Experiments have also indicated that IGF-1R is involved in the development and maintenance of metastatic phenotypes. In particular, high expression of a dominant negative mutant of IGF-1R (486stop) in ER positive breast cancer cells has been shown to inhibit adhesion, invasion, and metastasis of the cells (Dunn *et al.*, 1998, *Cancer Res.* **58**:3353-3361). Moreover, lung carcinoma cells exhibited an enhanced metastatic phenotype following overexpression of IGF-1R (Long *et al.*, 1998, *Exp. Cell Res.* **238**:116-121). In addition, activation of IGF-1R has been shown to block apoptotic pathways. Apoptosis in mammary glands was inhibited in IGF-1 transgenic mice (Hadsell *et al.*, 2000, *Oncogene* **19**:889-898). Moreover, down-regulation of IGF-1R function, either by antisense strategies or dominant negative mutants, caused massive apoptosis of tumor cells *in vitro* and *in vivo*. IGF-1 has also been shown to inhibit apoptosis associated with transformation by the c-myc oncogene and apoptosis induced by chemotherapeutic agents. The anti-apoptotic signaling of IGF-1 has been attributed to the PI3K-Akt

pathway, although other pathways may mediate similar effects (Butt *et al.*, 1999, *Immunol. Cell Biol.* **77**:256-262; B. Valentinis and R. Baserga, 2001, *Mol. Pathol.* **54**:133-137).

The sum of these observations indicate the importance of identifying antagonists or inhibitors of IGF-1 and/or IGF-1R. Attempts have been made to develop clinically relevant inhibitors of IGF-1R using monoclonal antibodies, antisense strategies, and peptide fragments derived from the natural ligand (Dunn *et al.*, 1998, *Cancer Res.* **58**:3353-3361; Z. Pietrzkowski *et al.*, 1992, *Cancer Res.* **52**:6447-6451; Z. Pietrzkowski *et al.*, 1993, *Cancer Res.* **53**:1102-1106; Rubini *et al.*, 1999, *Exp. Cell Res.* **251**:22-32). Using an alternate approach, this invention provides methods, kits, and compositions (e.g., pharmaceutical compositions) comprising IGF-1R antagonist peptides, or small molecule mimetics thereof, that can be useful in the diagnosis, treatment, and monitoring of one or more cancers. In some cases, the compositions, methods, and kits of the invention can also be used to determine the prognosis of a IGF-related medical condition (e.g., cancer). Advantageously, certain IGF-1R antagonist peptides disclosed herein are specific for Site 1 or Site 2 of the IGF-1 receptor.

In accordance with the invention, non-limiting cancer types include carcinoma, sarcoma, myeloma, leukemia, and lymphoma, and mixed types of cancers, such as adenosquamous carcinoma, mixed mesodermal tumor, carcinosarcoma, and teratocarcinoma. Representative cancers include, but are not limited to, bladder cancer, lung cancer, breast cancer, colon cancer, rectal cancer, endometrial cancer, ovarian cancer, head and neck cancer, prostate cancer, and melanoma. Specifically included are AIDS-related cancers (e.g., Kaposi's Sarcoma, AIDS-related lymphoma), bone cancers (e.g., osteosarcoma, malignant fibrous histiocytoma of bone, Ewing's Sarcoma, and related cancers), and hematologic/blood cancers (e.g., adult acute lymphoblastic leukemia, childhood acute lymphoblastic leukemia, adult acute myeloid leukemia, childhood acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, hairy cell leukemia,

cutaneous T-cell lymphoma, adult Hodgkin's disease, childhood Hodgkin's disease, Hodgkin's disease during pregnancy, mycosis fungoides, adult non-Hodgkin's lymphoma, childhood non-Hodgkin's lymphoma, non-Hodgkin's lymphoma during pregnancy, primary central nervous system lymphoma, Sezary syndrome, cutaneous T-cell lymphoma, Waldenström's macroglobulinemia, multiple myeloma/plasma cell neoplasm, myelodysplastic syndrome, and myeloproliferative disorders).

Also included are brain cancers (e.g., adult brain tumor, childhood brain stem glioma, childhood cerebellar astrocytoma, childhood cerebral astrocytoma, childhood ependymoma, childhood medulloblastoma, supratentorial primitive neuroectodermal and pineal, and childhood visual pathway and hypothalamic glioma), digestive/gastrointestinal cancers (e.g., anal cancer, extrahepatic bile duct cancer, gastrointestinal carcinoid tumor, colon cancer, esophageal cancer, gallbladder cancer, adult primary liver cancer, childhood liver cancer, pancreatic cancer, rectal cancer, small intestine cancer, and gastric cancer), musculoskeletal cancers (e.g., childhood rhabdomyosarcoma, adult soft tissue sarcoma, childhood soft tissue sarcoma, and uterine sarcoma), and endocrine cancers (e.g., adrenocortical carcinoma, gastrointestinal carcinoid tumor, islet cell carcinoma (endocrine pancreas), parathyroid cancer, pheochromocytoma, pituitary tumor, and thyroid cancer).

Further included are neurologic cancers (e.g., neuroblastoma, pituitary tumor, and primary central nervous system lymphoma), eye cancers (e.g., intraocular melanoma and retinoblastoma), genitourinary cancers (e.g., bladder cancer, kidney (renal cell) cancer, penile cancer, transitional cell renal pelvis and ureter cancer, testicular cancer, urethral cancer, Wilms' tumor and other childhood kidney tumors), respiratory/thoracic cancers (e.g., non-small cell lung cancer, small cell lung cancer, malignant mesothelioma, and malignant thymoma), germ cell cancers (e.g., childhood extracranial germ cell tumor and extragonadal germ cell tumor), skin cancers (e.g., melanoma, and merkel cell carcinoma),

gynecologic cancers (e.g., cervical cancer, endometrial cancer, gestational trophoblastic tumor, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, uterine sarcoma, vaginal cancer, and vulvar cancer), and unknown primary cancers.

Specific breast cancers include, but are not limited to, non-invasive cancers, such as ductal carcinoma *in situ* (DCIS), intraductal carcinoma lobular carcinoma *in situ* (LCIS), papillary carcinoma, and comedocarcinoma, or invasive cancers, such as adenocarcinomas, or carcinomas, e.g., infiltrating ductal carcinoma, infiltrating lobular carcinoma, infiltrating ductal and lobular carcinoma, medullary carcinoma, mucinous (colloid) carcinoma, comedocarcinoma, Paget's Disease, papillary carcinoma, tubular carcinoma, and inflammatory carcinoma. Specific prostate cancers may include adenocarcinomas and sarcomas, or pre-cancerous conditions, such as prostate intraepithelial neoplasia (PIN). Specific lung cancers include those relating to tumors such as bronchial carcinoid (bronchial adenoma), chondromatous hamartoma (benign), solitary lymphoma, and sarcoma (malignant) tumors, as well as lung cancers relating to multifocal lymphomas. Bronchogenic carcinomas may present as squamous cell carcinomas, small cell carcinomas, non-small cell carcinomas, or adenocarcinomas.

The IGF-1R antagonist peptides of the invention may be administered individually, or in combination with other IGF-1 or IGF-1R antagonists or inhibitors. Alternatively, the disclosed IGF-1R antagonist peptides can be used in combination with other cancer therapies, e.g., surgery, radiation, biological response modification, immunotherapy, hormone therapy, and/or chemotherapy. For prostate cancers, non-limiting examples of chemotherapeutic agents include docetaxel, paclitaxel, estramustine, etoposide, vinblastine, mitoxantrone, and paclitaxel. For breast cancers, non-limiting examples of chemotherapeutic and biological agents include cyclophosphamide, methotrexate, 5-fluorouracil, doxorubicin, tamoxifen, paclitaxel, docetaxel, navelbine, capecitabine, mitomycin C, Interferons,

interleukin-2, lymphocyte-activated killer cells, tumor necrosis factors, and monoclonal antibodies (e.g., mAb to HER-2/neu receptor (trastuzumab) Herceptin®). For lung cancers, non-limiting examples of chemotherapeutic and biological agents include, but are not limited to, platinum compounds (e.g., cisplatin or carboplatin), vinca alkaloids (e.g., vinorelbine, vincristine, or vinblastine), taxines (e.g., docetaxel or paclitaxel), and various topoisomerase inhibitors.

EXAMPLES

The examples as set forth herein are meant to exemplify the various aspects of the present invention and are not intended to limit the invention in any way.

The following materials were used in the examples described below. Soluble IGF-1R was obtained from R&D Systems (Minneapolis, MN; Cat. # 391-GR/CF). Insulin receptor was prepared according to Bass *et al.*, 1996. The insulin was either from Sigma (St. Louis, MO; Cat. # I-0259) or Boehringer. The IGF-1 was from PeproTech (Cat. # 100-11). All synthetic peptides were synthesized by Novo Nordisk, AnaSpec, Inc. (San Jose, CA), PeptioGenics (Livermore, CA), or Research Genetics (Huntsville, AL) at >80% purity. The Maxisorb Plates were from NUNC via Fisher (Cat. # 12565347). The HRP/Anti-M13 conjugate was from Pharmacia (Cat. # 27-9421-01). The ABTS solution was from BioF/X (Cat. # ABTS-0100-04).

Example 1: Monomer and Dimer Peptides

A. Cloning

Monomer and dimer peptides were constructed and expressed as protein fusions to a chitin binding domain (CBD) using the pTYB2 vector from the IMPACT™-CN system (New England Biolabs (NEB), Beverly, MA). The pTYB2 vector encodes a protein-splicing element (termed intein), which

initiates self-cleavage upon the addition of DTT. The intein self-cleavage separates the dimer from the affinity tag, to allow purification.

In the pTYB2 construct, the C-terminus of the peptide sequence was fused to the N-terminus of the intein/CBD sequence. Two peptide-flanking epitope tags were included: a shortened-FLAG® at the N-terminus and E-Tag at the C-terminus. This fusion was generated by ligating a vector fragment encoding the intein/CBD with a PCR product encoding the peptide of interest.

The vector fragment was obtained by digesting at appropriate restriction sites the pTBY2 vector. The digested DNA fragment was resolved on a 1% agarose gel, excised, and purified by QIAEXII (QIAGEN, Valencia, CA). To obtain the PCR product of the target proteins, primers were synthesized which anneal to appropriate sequences. The vector and insert were ligated overnight at 15°C. The ligation product was purified using QIAquick spin columns (QIAGEN) and electroporations were performed at 1500 V in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 10 ng of DNA and 40 µl of *E. coli* strain BL21.

Immediately following electroporation, 1 ml of pre-warmed (40°C) 2xYT medium containing 2% glucose (2xYT-G) was added to the transformants. The transformants were grown at 37°C for 1 h, and then plated onto 2xYT-AG plates and incubated overnight at 37°C. Individual colonies were isolated and used to innoculate 2xYT-G. The cultures were grown overnight at 37°C. Plasmid DNA was isolated from the cultures and sequencing was performed to confirm that the correct construct was obtained.

B. Small-scale expression of peptide-CBD fusion proteins

E .coli ER2566 (New England Biolabs) containing plasmids encoding peptide-CBD fusion proteins were grown in 2xYT-AG at 37°C overnight, with agitation (250 rpm). The following day, the cultures were used to inoculate media (2x YT-G) to obtain an OD_{600} of 0.1. Upon reaching an OD_{600} of 0.6,

expression of the fusion protein was induced by the addition of IPTG (isopropyl-β-D-thiogalactopyranoside) to a final concentration of 0.3 mM. Cells were grown for 3 h. Following this, cells were pelleted by centrifugation and the cell pellets were analyzed by SDS-PAGE electrophoresis. Production of the correct molecular weight fusion proteins was confirmed by Western blot analysis using the monoclonal antibody anti-E-Tag-HRP conjugate (Amersham Pharmacia).

C. Large-scale expression and purification of soluble peptide-CBD fusion proteins

E. coli ER2566 carrying plasmids encoding the fusion proteins were grown in 2xYT-AG media at 37°C for 8 h, with agitation (250 rpm). The cultures were back-diluted into to 2 L volumes of 2xYT-A to achieve an OD_{600} of 0.1. Upon reaching an OD_{600} of 0.5, IPTG was added to a final concentration of 0.3 mM. Cells were grown at 30°C overnight. The next day cells were isolated by centrifugation. Samples of the cell pellet were analyzed by SDS-PAGE followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product.

D. Purification

The cell pellets were disrupted mechanically by sonication or chemically by treatment with the mild detergent. After removal of cell debris by centrifugation, the soluble proteins in the clarified lysate were prepared for chromatographic purification by dilution or dialysis into the appropriate starting buffer. The CBD fusions were purified by chitin affinity chromatography according to the manufacturer's instructions (New England Biolabs). The lysate was loaded onto a chitin affinity column and the column was washed with 10 volumes of column buffer. Three bed volumes of the DTT containing cleavage buffer were loaded onto the column and the column was incubated overnight. The next day, the target protein was

eluted by continuing the flow of the cleavage buffer without DTT. The purified proteins were analyzed for purity and integrity by SDS-PAGE and Western blot analysis according to standard protocols.

Example 2: PEG-Based Dimer Peptides

A. Synthesis of the aldehyde containing peptide

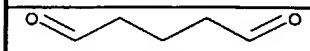
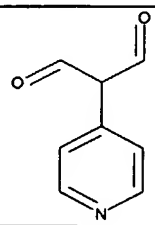
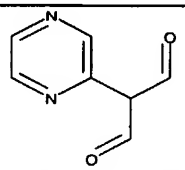
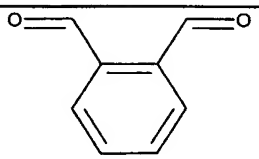
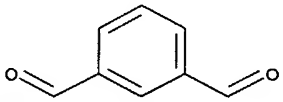
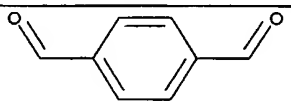
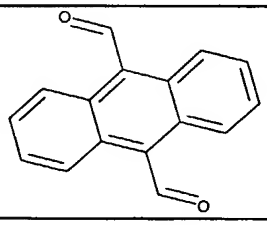
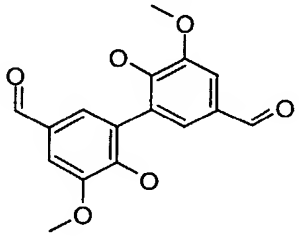
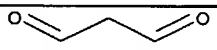
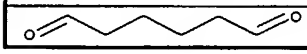
The peptide was synthesized by stepwise solid phase synthesis on Rink amide Tentagel (0.21 mmol/g). Three equivalents of Fmoc-amino acids were used. The serine residue was introduced into the peptide by either coupling Fmoc-Ser(tBu)-OH to the N-terminal peptide or coupling Boc-Ser(tBu) to a selectively protected lysine side-chain. The peptide was then deprotected and cleaved from the resin by treatment with 95% TFA (trifluoroacetic acid; aq) containing TIS (triisopropylsilan). Periodate oxidation, using 2 equivalent of $NaIO_4$ in 20% DMSO (dimethyl sulfoxide)-80% phosphate buffer pH 7.5 (45 μl/μmol peptide) for 5 min at room temperature (RT), converted the 2-amino alcohol moiety in an α-oxoacyl group. The peptide was purified immediately following oxidation.

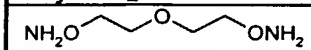
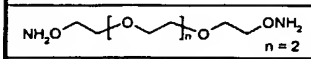
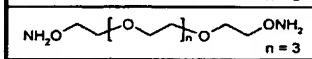
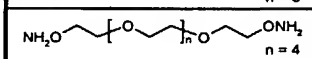
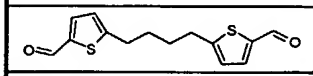
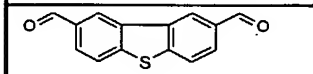
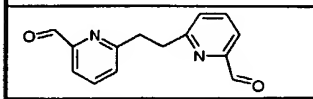
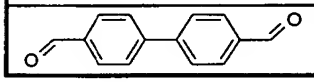
B. Synthesis of the PEG-based dimer

The unprotected and oxidized peptide (4.2 equivalent) was dimerized on the dioxyamino-PEG (polyethylene glycol)-linker (1 equivalent) in 90% DMSO-10% 20 mM NaOAc buffer, pH 5.1 (4.2 μl/μmol peptide). The solution was left for 1 h at 38°C and the progress of the reaction was monitored by MALDI-MS (matrix-assisted laser desorption/ionization mass spectrometry). Following this, the crude dimer was purified by semi-preparative HPLC (high performance liquid chromatography).

The molecular weights and inter peptide distance of various linkers is shown in Table 3, below.

TABLE 3

Structure	Number	MW	MW (- $2H_2O$)
	1	100.1	64.1
	2	58.04	22.04
	3	149.15	113.15
	4	150.14	114.14
	5	134.13	98.13
	6	134.13	98.13
	7	134.13	98.13
	8	234.25	198.25
	9	302.3	266.3
	10	72.06	36.06
	11	86.09	50.09
	12	114.14	78.14

	13	128.08	92.08
	14	142.19	106.19
$(HCO)_4$-$(Lys)_2$-Lys-Gly-NH_2	15		
	16	136.2	100.2
	17	180.2	144.2
	18	224.3	188.3
	19	268.3	232.3
	20	312.4	276.4
	21	278.4	242.4
	22	240.3	204.3
	23	240.3	204.3
	24	210.2	192.2

Example 3: Determination of Insulin Receptor Binding

IR was incubated with ^{125}I-labeled insulin at various concentrations of test substance and the K_d was calculated. According to this method, human insulin receptor (HIR) or human IGF-1 receptor (HIGF-1R) was purified from transfected cells after solubilization with Triton X-100. The assay buffer contained 100 mM HEPES (pH 7.8), 100 mM NaCl, 10 mM $MgCl_2$, 0.5% human serum albumin (HSA), 0.2% gammaglobulin and 0.025% Triton X-100. The receptor concentration was chosen to give 30-60% binding of 2000 cpm (3 pM) of its ^{125}I-labeled ligand (TyrA14-^{125}I-HI or Tyr31-^{125}I-IGF1) and a dilution series of the substance to be tested was added. After equilibration for 2 days at 4°C, each sample (200 µl) was precipitated by addition of 400 µl 25% PEG 6000, centrifuged, washed with 1 ml 15% PEG 6000, and counted in a gamma-counter.

The insulin/IGF-1 competition curve was fitted to a one-site binding model and the calculated parameters for receptor concentration, insulin

affinity, and non-specific binding were used in calculating the binding constants of the test substances. Representative curves for insulin competition are shown in Figures 10A-10C; 11A-11D. Qualitative data are provided in Table 4, below.

Table 4 illustrates IR affinities for the RP9 monomer peptide and various RP9 monomer truncations. The results demonstrate that RP9 N-terminal sequence (GSLD; SEQ ID NO:1785) and C-terminal sequence (LGKK; SEQ ID NO:1786) can be deleted without substantially affecting HIR binding affinity (Table 4).

TABLE 4

Peptide	SEQ ID NO:	Formula	Site IR	Sequence	HIR Kd (mol/l)
S386	1559	1	1	GSLDESFYDWFERQLG	$3.2*10^{-7}$
S395	1787	1	1	GSLDESFYDWFERQL	$9.1*10^{-8}$
S394	1788	1	1	GSLDESFYDWFERQ	$8.1*10^{-8}$
S396	1789	1	1	GSLDESFYDWFER	$>2*10^{-5}$
S399	1790	1	1	ESFYDWFERQL	$9.1*10^{-8}$
S400	1791	1	1	ESFYDWFERQ	$6.3*10^{-7}$

Figures 10A-10C demonstrate that Site 1-Site 2 heterodimer peptides 537, 538, and 539 bound to IR with substantially higher (several orders of magnitude) affinity than corresponding monomer (D117 and 540) and homodimer (521 and 535) peptides. Figures 11A-11D demonstrate that Site 1-Site 2 heterodimer peptides, 537 and 538, bound to IR with markedly higher affinity than the monomer peptide D117.

Example 4: Adipocyte Assay for Determination of Insulin Agonist Activity

Insulin increases uptake of ^{3}H glucose into adipocytes and its conversion into lipid. Incorporation of ^{3}H into the lipid phase was determined by partitioning of lipid phase into a scintillant mixture, which excludes water-soluble ^{3}H products. The effect of compounds on the incorporation of ^{3}H glucose at a sub-maximal insulin dose was determined, and the results

expressed as increase relative to full insulin response. The method was adapted from Moody *et al.*, 1974, *Horm. Metab. Res.* **6**(1):12-6.

Mouse epididymal fat pads were dissected out, minced into digestion buffer (Krebs-Ringer 25 mM HEPES, 4% HSA, 1.1 mM glucose, 0.4 mg/ml Collagenase Type 1, pH 7.4), and digested for up to 1.5 h at 36.5°C. After filtration, washing (Krebs-Ringer HEPES, 1% HSA), and resuspension in assay buffer (Krebs-Ringer HEPES, 1% HSA), free fat cells were pipetted into 96-well Picoplates (Packard), containing test solution and approximately an ED_{20} insulin.

The assay was started by addition of ^3H glucose (Amersham TRK 239), in a final concentration of 0.45 mM glucose. The assay was incubated for 2 h, 36.5°C, in a Labshaker incubation tower, 400 rpm, then terminated by the addition of Permablend/Toluene scintillant (or equivalent), and the plates sealed, before standing for at least 1 h and detection in a Packard Top Counter or equivalent. A full insulin standard curve (8 dose) was run as control on each plate.

Data are presented graphically, as effect of compound on an (approximate) ED_{20} insulin response, with data normalized to a full insulin response. The assay can also be run at basal or maximal insulin concentration. Representative dose-response curves for insulin and IGF-1 are shown in Figures 12-18. Qualitative data are shown in Tables 5-7.

In free fat cell (FFC) assays, truncated synthetic RP9 monomer peptides S390 and S394 showed potency similar to full-length RP9 monomer peptides (Figures 12A-12D). Truncated synthetic RP9 homodimer peptides S415 and S417 were highly potent in FFC assays, but less potent than full-length RP9 homodimer peptides (Figures 13A-13C; compare to peptides 521 and 535, described below). The potency of recombinant RP9 homodimer peptides 521 and 535 in FFC assays is shown in Figures 14A-14C. The curves are flattened, suggesting that the binding mechanism may not be mediated by simple intramolecular binding (Figures 14A-14C).

Results further indicated that synthetic RP9 homodimer peptides S337 and S374 showed increased HIR biding affinity and increased potency in FFC assays compared to synthetic RP9 monomer, S371 (Table 5). Similarly, synthetic RP9 homodimer peptides S314 and S317 showed increased HIR binding affinity and increased potency in FFC assays compared to synthetic RP9 monomer, S371, and various RP9 truncations (Table 6).

TABLE 5

Pep.	SEQ ID NO:	Formula	Site IR	Monomer or Dimer	Sequence	HIR K_d (mol/l)	FFC
S371	1558	1	1	M (RP9)	GSLDESFYDWFERQLGKK	$6.3.*10^{-7}$	+
S337	1792	1-1	1-1	D, C-Term 23	$(GSLDESFYDWFERQLGKK\text{-}Lig)_2$-23	$1.1*10^{-8}$	+++++
S374	1793	1-1	1-1	D, N-Term 17	17-$(GSLDESFYDWFERQLGKK)_2$	$1.8*10^{-7}$	++++

M = monomer; D = dimer; C-Term = C-terminal linker (C-C); N-Term = N-terminal linker (N-N); 23 and 17 represent specific chemical linkers (see Table 3); For FFC: 0 is no effect, + is agonist, - is antagonist.

TABLE 6

Peptide	SEQ ID NO:	Form.	Site IR	Mon. or Dimer	Sequence	HIR K_d (mol/l)	FFC
S371 (RP9)	1558	1	1	M	GSLDESFYDWFERQLGKK	$6.3.*10^{-7}$	+
S395	1787	1	1	M	GSLDESFYDWFERQL	$9.1*10^{-8}$	+
S394	1788	1	1	M	GSLDESFYDWFERQ	$8.1*10^{-8}$	++
S396	1789	1	1	M	GSLDESFYDWFER	$>2*10^{-5}$	0
S390	1794	1	1	M	ESFYDWFERQLG	$6.2*10^{-7}$	+
S399	1790	1	1	M	ESFYDWFERQL	$9.1*10^{-8}$	++
S400	1791	1	1	M	ESFYDWFERQ	$6.3*10^{-7}$	0
S415	1795	1-1	1-1	D; C-Term	$(ESFYDWFERQLGK)_2$-23	$1.0*10^{-7}$	++++
S417	1796	1-1	1-1	D; N-Term	23-$(ESFYDWFERQLG)_2$	$9.2*10^{-7}$	+++

M = monomer; D = dimer; C-Term = C-terminal linker (C-C); N-Term = N-terminal linker (N-N); 23 represents a specific chemical linker (see Table 3); For FFC: 0 is no effect, + is agonist, - is antagonist; Form. = formula; Mon. = monomer;

Site 1-Site 2 dimer peptides 537 and 538 were inactive in the FFC assays using the standard concentration of insulin (Figures 15A-15C). However, Site 1-Site 2 dimer peptides 537 and 538 were antagonists in the

FFC assay in the presence of a stimulating concentration of insulin (Figures 16A-16C). In contrast, Site 2-Site 1 dimer peptide 539 was a full agonist in the FFC assay, with a slope similar to that of insulin (Figures 17A-17B).

Additional experiments confirmed that FFC assay activity of Site 1-Site 2 dimer peptides was affected by the orientation of the monomer subunits (Figures 18A-18D). In particular, dimer peptides comprising Site 1 (S372 or S373) and Site 2 (S451 or S452) monomer subunits exhibited antagonist activity in the Site 1-Site 2 orientation (C-N linkage) (dimer peptide S453); moderate levels of agonist activity in the Site 1-Site 2 orientation (N-N or C-C linkage) (dimer peptides S454 and S456); and high levels of agonist activity in the Site 2-Site 1 orientation (C-N linkage) (dimer peptide S455) (Figures 18A-18D).

Table 7, below, shows the HIR binding affinity and FFC assay potency of various synthetic peptides, including Site 1-Site 1 dimer peptides S325, S329, S332; S333, S334, S335, S336, S337, S349, S350, S351, S352, S353, S354, S361, S362, S363, S374, S375, S376, S378, S379, S380, S381, S414, S415, S416, S417, S418, S420, and S424. These synthetic dimer peptides exhibited properties comparable to dimer peptides 521 and 535, regardless of the orientation of the monomer subunits. In particular, synthetic Site 1-Site 2 dimer peptides S425, S453, and S459 exhibited antagonist properties comparable to those of the Site 1-Site 2 dimer peptides 537 and 538. Synthetic Site 1-Site 2 dimer peptides S455, S457, and S458 exhibited agonist properties comparable to the dimer peptide 539. Synthetic Site 1-Site 2 dimer peptides S436, S437, S438, S454, S456 act as partial agonists in the FFC assay (i.e., the peptides exhibit a maximal response of less than 100% that of insulin), which is shown in the table as "++" and "+++".

Table 7 also shows properties of truncated monomer and dimer peptides, and thereby indicates which N- or C-terminal residues can be deleted without substantial loss of HIR binding affinity (e.g., see synthetic peptides S386 through S392, S394 through S403, and S436 through S445).

Notably, certain Site 2-Site 1 dimers show IR affinities of $2*10^{-11}$ (see, e.g., S519 and S520). These peptides are also very potent in the fat cell assay (Figures 31A-31B) and even more potent in the HIR kinase assay (Figures 32A-32B) (kinase assay described below).

TABLE 7

Peptide	SEQ ID NO:	Formula	Linkage	Site IR	Sequence	HIR Kd (mol/l)	FFC
S105	1797	F1	-	1	FHENFYDWFVRQVAKK	$3.1*10^{-7}$	++
S106	1798	F1	-	1	FHENFYDWFVRQASKK	$4.2*10^{-7}$	++
S107	1799	F1	-	1	FHENFYDWFVRAVSKK	$10.0*10^{-7}$	+
S108	1800	F1	-	1	FHENFYDWFVAQVSKK	$7.5*10^{-7}$	+
S109	1801	F1	-	1	FHENFYDWFARQVSKK	$2.3*10^{-7}$	++
S110	1802	F1	-	1	FHEAFYDWFVRQVSKK	$2.2*10^{-7}$	++
S111	1803	F1	-	1	FHANFYDWFVRQVSKK	$3.3*10^{-7}$	0
S112	1804	F1	-	1	FAENFYDWFVRQVSKK	$6.1*10^{-7}$	+
S113	1805	F1	-	1	AHENFYDWFVRQVSKK	$5.9*10^{-7}$	+
S114	1556	F1	-		fhenfydwfvrqvskk	$8.3*10^{-6}$	0
S115	1806	F1	-	1	EFHENFYDWFVRQVSEE	$6.5*10^{-7}$	+
S116	1807	F1	-	1	FHENFYGWFVRQVSKK	$1.4*10^{-6}$	++
S117	1808	F2	-	1	HETFYSMIRSLAK	$2.7*10^{-6}$	0
S118	1809	F2	-	1	SDGFYNAIELLS	$2.4*10^{-6}$	+
S119	1810	F2	-	1	SLNFYDALQLLAKK	$1.8*10^{-6}$	0
S120	1811	F2	-	1	HDPFYSMMKSLLK	$2.0*10^{-6}$	0
S121	1812	F2	-	1	NSFYEALRMLSSK	$3.1*10^{-6}$	0
S122	1813	F7	-		HPTSKEIYAKLLK	$9.3*10^{-6}$	0
S123	1814	F7	-		HPSTNQMLMKLFK	$1.6*10^{-5}$	0
S124	1815	F7	-		HPPLSELKLFLIKK	$2.3*10^{-5}$	0
S127	1816	F2	-	1	WSDFYSYFQGLD	$1.2*10^{-6}$	0
S128	1817 and 1818	F1-F1	C-C	1-1	$(FHENFYDWFVRQVSKK)_2$-Dap	$1.1*10^{-6}$	++
S129	1819	F2	-	1	SSNFYQALMLLS	$2.9*10^{-6}$	0

S131	1820	F1	-	1	FHENFYDWFVRQVSKK-Lig	$1.2*10^{-6}$	+
S137	1821	F1	-	1	HENFYGWFVRQVSKK	$7.7*10^{-7}$	0
S145	1822 and 1823	F1-F1	C-C	1-1	$(FHENFYDWFVRQVSKK)_2$-Lys	$1.5*10^{-6}$	++
S158	1780	F1	-	1	FHENFYDWFVRQVSK	$8.1*10^{-7}$	+
S165	1554	F1	-	1	FYDWF	$>2*10^{-5}$	0
S166	1824	F1	-	1	FYDWFKK	$>2*10^{-5}$	0
S167	1825	F1	-	1	AFYDWFAKK	$>2*10^{-5}$	-
S168	1826	F1	-	1	AAAAFYDWFAAAAAKK	$3.8*10^{-6}$	0
S169	1827 and 1828	F1-F1	N-N	1-1	$12\text{-}(Lig\text{-}FHENFYDWFVRQVSKK)_2$	$5.8*10^{-7}$	++
S170	1829 and 1830	F1-F1	N-N	1-1	$(CGFHENFYDWFVRQVSKK)_2$ (linked at cysteines)	$7.0*10^{-7}$	+++
S171	1831	F1	-	1	CGFHENFYDWFVRQVSKK	$2.9*10^{-6}$	+++
S172	1832 and 1833	F1-F1	N-N	1-1	$14\text{-}(Lig\text{-}FHENFYDWFVRQVSKK)_2$	$4.8*10^{-6}$	+++
S173	1834	F3	-	1	LDALDRLMRYFEERPSL	$1.2*10^{-6}$	0
S174	1835	F3	-	1	PLAELWAYFEHSEQGRSSAH	$1.6*10^{-5}$	0
S175	1560	F1	-	1	GRVDWLQRNANFYDWFVAELG	$2.3*10^{-7}$	+++
S176	1836	F1	-	1	NGVERAGTGDNFYDWFVAQLH	$4.7*10^{-7}$	+
S177	1837	F2	-	1	EHWNTVDPFYFTLFEWLRESG	$2.7*10^{-6}$	0
S178	1838	F2	-	1	EHWNTVDPFYQYFSELLRESG	$1.3*10^{-7}$	++
S179	1839	F1	-	1	QSDSGTVHDRFYGWFRDTWAS	$5.4*10^{-7}$	+
S180	1840	F1	-	1	AFYDWFAK	$>2*10^{-5}$	0
S181	1841	F1	-	1	AFYDWFA	$>2*10^{-5}$	0
S182	1842	F1	-	1	AFYDWF	$>2*10^{-5}$	0
S183	1843	F1	-	1	FYDWFA	$>2*10^{-5}$	0
S184	1844	F1	-	1	Ac-FYDWF	$>2*10^{-5}$	0
S214	1845	F1	-	1	AFYEWFAKK	$>2*10^{-5}$	0
S215	1846	F1	-	1	AFYGWFAKK	$>2*10^{-5}$	0
S216	1847	F1	-	1	AFYKWFAKK	$>2*10^{-5}$	0

S217	1848 and 1849	F2-F2	C-C	1-1	$(SDGFYNAIELLS\text{-}Lig)_2$-14	$3.9*10^{-8}$	++
S218	1850 and 1851	F1-F1	C-C	1-1	$(AFYDWFAKK\text{-}Lig)_2$-14	$1.1*10^{-5}$	0
S219	1852	F1	-	1	FHENAYDWFVRQVSKK	$>2*10^{-5}$	0
S220	1853	F1	-	1	FHENFADWFVRQVSKK	$>2*10^{-5}$	0
S221	1854	F1	-	1	FHENFYAWFVRQVSKK	$1.1*10^{-6}$	+
S222	1855	F1	-	1	FHENFYDAFVRQVSKK	$>2*10^{-5}$	0
S223	1856	F1	-	1	FHENFYDWAVRQVSKK	$>2*10^{-5}$	0
S226	1857	F6	-	2	QLEEEWAGVQCEVYGRECPS	$1.6*10^{-6}$	
S227	1858	F1	-	1	CGGFHENFYDWFVRQVSKK	$5.1*10^{-7}$	++
S228	1859 and 1860	F1-F1	N-N	1-1	$(CGGFHENFYDWFVRQVSKK)_2$ **(linked at cysteines)**	$3.6*10^{-7}$	++
S229	1861 and 1862	F2-F4	C-C	1-2	SDGFYNAIELLS-Lig 12 KHLCVLEELFWGASLFGYCSGKK-Lig	$4.4*10^{-9}$	0
S231	1863 and 1864	F1-F1	C-C	1-1	$(FHENFYDWFVRQVSKKGGG\text{-}Lig)_2$-14	$2.7*10^{-7}$	+
S232	1865 and 1866	F1-F1	N-N	1-1	14-$(Lig\text{-}GGGFHENFYDWFVRQVSKK)_2$	$3.8*10^{-7}$	+++
S233	1867 and 1868	F1-F2	C-C	1-1	FHENFYDWFVRQVSKK-Lig 14 SDGFYNAIELLS-Lig	$2.6*10^{-7}$	+
S234	1869	F1	-	1	RVDWLQRNANFYDWFVAELG	$1.3*10^{-7}$	++
S235	1870	F1	-	1	VDWLQRNANFYDWFVAELG	$5.3*10^{-8}$	++
S236	1871	F1	-	1	DWLQRNANFYDWFVAELG	$1.0*10^{-7}$	++
S237	1872	F1	-	1	WLQRNANFYDWFVAELG	$8.5*10^{-7}$	0
S238	1873	F1	-	1	LQRNANFYDWFVAELG	$8.5*10^{-7}$	0
S239	1874	F1	-	1	QRNANFYDWFVAELG	$1.3*10^{-6}$	0
S240	1875	F1	-	1	RNANFYDWFVAELG	$1.4*10^{-6}$	
S241	1876	F1	-	1	NANFYDWFVAELG	$1.6*10^{-6}$	
S242	1877	F1	-	1	ANFYDWFVAELG	$2.0*10^{-6}$	

S243	1878	F1	-	1	NFYDWFVAELG	$2.0*10^{-6}$	
S244	1879	F1	-	1	GRVDWLQRNANFYDWFVAELG-Lig	$2.2*10^{-7}$	++
S245	1880	F1	-	1	Lig-GRVDWLQRNANFYDWFVAELG	$2.2*10^{-7}$	+
S246	1881 and 1882	F8-F1	C-C	3-1	ACAWPTYWNCGGGG-Lig 14 FHENFYDWFVRQVSKK-Lig	$5.0*10^{-6}$	
S248	1883	F1	-	1	GRVDWLQRNANFYDWFVAEL	$6.3*10^{-8}$	++
S249	1884	F1	-	1	GRVDWLQRNANFYDWFVAE	$7.4*10^{-7}$	0
S250	1885	F1	-	1	GRVDWLQRNANFYDWFVA	$8.9*10^{-6}$	0
S251	1886	F1	-	1	GRVDWLQRNANFYDWFV	$5.6*10^{-6}$	
S252	1887 and 1888	F2-F2	C-C	1-1	(SDGFYNAIELLS-Lig)$_2$-14	$4.4*10^{-7}$	0
S253	1889 and 1890	F1-F1	C-C	1-1	(GRVDWLQRNANFYDWFVAELG-Lig)$_2$-14	$2.2*10^{-8}$	++
S255	1891 and 1892	F2-F2	C-C	1-1	(SDGFYNAIELLSGGG-Lig)$_2$-14	$1.6*10^{-6}$	0
S256	1893	F6	-	2	Acy-CLEEwGASL-Tic-QCSG	$9.0*10^{-6}$	-
S257	1894	F2	-	1	RWPNFYGYFESLLTHFS	$1.4*10^{-5}$	0
S259	1895	F2	-	1	EGWDFYSYFSGLLASVT	$7.7*10^{-6}$	0
S260	1896	F2	-	1	LDRQFYRYFQDLLVGFM	$2.3*10^{-6}$	0
S261	1897	F2	-	1	WGRSFYRYFETLLAQGI	$>2*10^{-5}$	0
S262	1898	F4	-	1	PLCFLQELFGGASLGGYCSG	$1.9*10^{-5}$	0
S263	1899	F6	-	2	WLEQERAWIWCEIQGSGCRA	$>2*10^{-5}$	0
S264	1900	F1	-	1	IQGWEPFYGWFDDVVAQMFEE	$1.9*10^{-7}$	0
S265	1901	F1	-	1	TGHRLGLDEQFYWWFRDALSG	$1.1*10^{-7}$	0
S266	1902	F6	-	2	Abu-CLEEwGASL-Tic-QCSG	$>2*10^{-5}$	0
S268	1903	F1	-	1	RD-Hyp-FYDWFDDi	$4.5*10^{-7}$	0
S273	1904	F1-F2	C-N	1-1	FHENFYDWFVRQVSKK-Lig-14-Lig-SDGFYNAIELLS	$1.5*10^{-6}$	+
S278	1905	F1-derived	-	1	GFREGQRWYWFVAQVT	$>2*10^{-5}$	0
S281	1906	F5	-		DLRVLCELFGGAYVLGYCSE	$1.1*10^{-5}$	0

S282	1907	F4-derived	-		HLSVGEELSWWVALLGQWAR	>2*10^{-5}	0
S283	1908	F4-derived	-		APVSTEELRWGALLFGQWAG	>2*10^{-5}	0
S284	1909	F6-derived	-		ALEEEWAWVQVRSIRSGLPL	>2*10^{-5}	0
S285	1910	F6-derived	-		WLEHEWAQIQCELYGRGCTY	8.3*10^{-7}	
S287	1911	F1	-	1	QAPSNFYDWFVREWDEE	5.9*10^{-6}	0
S288	1912	F2	-	1	QSFYDYIEELLGGEWKK	4.3*10^{-6}	0
S289	1913	F2	-	1	DPFYQGLWEWLRESGEE	>2*10^{-5}	0
S290	1914 and 1915	F1-F1	N-N	1-1	7–(Lig-GGGFHENFYDWFVRQVSKK)$_2$	9.0*10^{-7}	++
S291	1916 and 1917	F1-F1	N-N	1-1	9-(Lig-GGGFHENFYDWFVRQVSKK)$_2$	1.2*10^{-6}	++++
S292	1918 and 1919	F1-F1	N-N	1-1	12-(Lig-GGGFHENFYDWFVRQVSKK)$_2$	7.5*10^{-7}	++
S293	1920 and 1921	F1-F1	N-N	1-1	13-(Lig-GGGFHENFYDWFVRQVSKK)$_2$	1.2*10^{-7}	++
S294	1922	F1	-	1	DWLQRNANFYDWFVAEL-Lig	1.3*10^{-7}	++
S295	1923	F1	-	1	Lig-DWLQRNANFYDWFVAEL	4.8*10^{-7}	+
S300	1924 and 1925	F1-F1	C-C	1-1	(DWLQRNANFYDWFVAEL-Lig')$_2$-14	5.0*10^{-8}	+++
S301	1926 and 1927	F1-F1	N-N	1-1	14-(Lig'-DWLQRNANFYDWFVAEL)$_2$	6.4*10^{-7}	+
S302	1928	F2	-	1	SDGFYNA-Acy-ELLSG	8.6*10^{-7}	0
S303	1929	F2	-	1	SGPFYEE-Acy-ELLW-Aib-G	5.7*10^{-6}	0
S304	1930	F2	-	1	GGSFYDD-Acy-E-Aib-LW-Aib-G	2.1*10^{-5}	0
S305	1931	F2	-	1	N-Aib-PFYDE-Acy-DE-Cha-W-Aib-G	8.4*10^{-7}	0
S306	1932	F1	-	1	GRVDWLQRNANFYDWFVAE-Acy-G	2.2*10^{-6}	+++
S312	1933 and 1934	F1-F1	N-N	1-1	23-(Lig'-GGGFHENFYDWFVRQVSKK)$_2$	2.9*10^{-6}	++
S313	1935 and 1936	F2-F2	C-C	1-1	(SDGFYNAIELLS-Lig')$_2$-23	2.4*10^{-7}	
S315	1937	F1	-	1	WFYDWFWE	6.8*10^{-6}	0
S316	1938	F10	-	1	WQGYAWLS	7.0*10^{-6}	0
S317	1939	F10	-	1	WPGYAWLS	>2*10^{-5}	0
S319	1940	F1	-	1	D-Aic-D-Aib-EFYDWFDEiPq	8.7*10^{-7}	0
S320	1941	F1	-	1	KNNKEFYEWFDEiGq	2.8*10^{-6}	0
S321	1942	F1	-	1	YeRD-Hyp-FYDWFDEiGq	1.4*10^{-6}	0
S322	1943	F1	-	1	EWRD-Hyp-FYDWFDEi-Hyp-e	7.2*10^{-7}	0
S325	1944 and 1945	F1-F1	N-N	1-1	9-(Lig'-GSLDESFYDWFERQLGKK)$_2$	4.6*10^{-8}	+++++

S326	1600	F1	-	1	GIISQSCPESFYDWFAGQVSDPWWCW	$5.9*10^{-7}$	-
S327	1946	F2	-	1	TFYSCLASLLTGTPQPNRGPWERCRKK	$2.1*10^{-6}$	-
S329	1947 and 1948	F1-F1	N-N	1-1	17-(Lig'-FHENFYDWFVRQVSKK)$_2$	$2.7*10^{-6}$	++
S331	1949	F4	-	2	KHLCVLEELFWGASLFGYCSGKK	$1.6*10^{-6}$	0
S332	1950 and 1951	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKK-Lig')$_2$-9	$2.1*10^{-8}$	+++++
S333	1952 and 1953	F1-F1	N-N	1-1	22-(Lig'-GSLDESFYDWFERQLGKK)$_2$	$1.4*10^{-7}$	++++
S334	1954 and 1955	F1-F1	N-N	1-1	22-(Lig'-GGGFHENFYDWFVRQVSKK)$_2$	$1.6*10^{-6}$	+++
S335	1956 and 1957	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKK-Lig')$_2$-22	$9.8*10^{-8}$	++++
S336	1958 and 1959	F1-F1	N-N	1-1	23-(Lig'-GSLDESFYDWFERQLGKK)$_2$	$1.5*10^{-8}$	+++
S337	1960 and 1961	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKK-Lig')$_2$-23	$1.1*10^{-8}$	+++++
S342	1962	F1	-	1	DLWFNAKEDMNFYDWFVWQLR	$1.8*10^{-6}$	0
S344	1963	F2	-	1	EHWNTVDPFYHWISELLRESGA	$2.0*10^{-7}$	0
S345	1964	F2	-	1	EHWNTVDPFYQYFAELLRESGA	$2.9*10^{-6}$	0
S349	1965 and 1966	F1-F1	N-N	1-1	23-(Lig'-GGGFHENFYDWFVRQVSKK)$_2$	$1.3*10^{-7}$	++++
S350	1967 and 1968	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKK-Lig')$_2$-21	$4.7*10^{-7}$	++++
S351	1969 and 1970	F1-F1	N-N	1-1	21-(Lig'-GSLDESFYDWFERQLGKK)$_2$	$1.4*10^{-6}$	+++
S352	1971 and 1972	F1-F1	N-N	1-1	21-(Lig'-GGGFHENFYDWFVRQVSKK)$_2$	$6.6*10^{-7}$	+++
S353	1973 and 1974	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKK-Lig')$_2$-14	$1.1*10^{-8}$	++++++
S354	1975 and 1976	F1-F1	N-N	1-1	14-(Lig'-GSLDESFYDWFERQLGKK)$_2$	$3.9*10^{-8}$	++++
S359	1977 and 1978	F1-F1	N-N	1-1	9-(Lig'-DWLQRNANFYDWFVAEL)$_2$	$7.0*10^{-7}$	+
S360	1979 and 1980	F1-F1	N-N	1-1	23-(Lig'-DWLQRNANFYDWFVAEL)$_2$	$9.9*10^{-7}$	
S361	1981 and 1982	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKK-Lig')$_2$-24	$2.2*10^{-6}$	+++
S362	1983 and 1984	F1-F1	N-N	1-1	24-(Lig'-GSLDESFYDWFERQLGKK)$_2$	$1.1*10^{-7}$	++++
S363	1985 and 1986	F1-F1	N-N	1-1	24-(Lig'-GGGFHENFYDWFVRQVSKK)$_2$	$2.2*10^{-7}$	+++
S365	1987	F1	-	1	RMYFSTGAPQNFYDWFVQEWD	$1.0*10^{-5}$	0
S366	1988	F1	-	1	PLRESRNFYDWFVQQLE	$3.7*10^{-7}$	0
S368	1989	F2	-	1	RGTRSDPFYHKLSELLQGH	$>2*10^{-5}$	0
S371	1558	F1	-	1	GSLDESFYDWFERQLGKK	$6.3.*10^{-7}$	+
S372	1990	F1	-	1	SGSLDESFYDWFERQLGKK	$2.0*10^{-7}$	++
S373	1991	F1	-	1	GSLDESFYDWFERQLGKKK(S)	$1.2*10^{-7}$	+++
S374	1992 and 1993	F1-F1	N-N	1-1	17-(Ald-GSLDESFYDWFERQLGKK)$_2$	$1.8*10^{-7}$	++++
S375	1994	F1-F1	C-N	1-1	(GSLDESFYDWFERQLGKKK-Ald)-14-(Ald-GSLDESFYDWFERQLGKK)	$2.0*10^{-7}$	++++

S376	1995 and 1996	F1-F1	N-N	1-1	19-(Ald-GSLDESFYDWFERQLGKK)$_2$	$1.6*10^{-7}$	++++
S378	1997 and 1998	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKKK-Ald)$_2$-17	$6.5*10^{-8}$	+++++
S379	1999 and 2000	F1-F1	C-C	1-1	(GSLDESFYDWFERQLGKKK-Ald)$_2$-19	$5.6*10^{-8}$	+++++
S380	2001 and 2002	F1-F1	C-C	1-1	(EEDWLQRNANFYDWFVAEL-Lig')$_2$-9	$5.1*10^{-7}$	++
S381	2003 and 2004	F1-F1	C-C	1-1	(EEDWLQRNANFYDWFVAEL-Lig')$_2$-23	$1.2*10^{-7}$	++++
S386	1559	F1	-	1	GSLDESFYDWFERQLG	$3.2*10^{-7}$	+
S387	2005	F1	-	1	SLDESFYDWFERQLG	$6.3*10^{-7}$	+
S388	2006	F1	-	1	LDESFYDWFERQLG	$3.4*10^{-7}$	+
S389	2007	F1	-	1	DESFYDWFERQLG	$1.1*10^{-6}$	+
S390	1794	F1	-	1	ESFYDWFERQLG	$6.2*10^{-7}$	+
S391	2008	F1	-	1	SFYDWFERQLG	$1.5*10^{-6}$	+
S392	2009	F1	-	1	FYDWFERQLG	$3.8*10^{-6}$	0
S394	1788	F1	-	1	GSLDESFYDWFERQ	$9.1*10^{-8}$	+
S395	1787	F1	-	1	GSLDESFYDWFERQL	$8.1*10^{-8}$	++
S396	1789	F1	-	1	GSLDESFYDWFER	$>2*10^{-5}$	0
S397	2010	F1	-	1	GSLDESFYDWFE	$>2*10^{-5}$	0
S398	2011	F1	-	1	GSLDESFYDWF	$>2*10^{-5}$	0
S399	1790	F1	-	1	ESFYDWFERQL	$9.5*10^{-8}$	++
S400	1791	F1	-	1	ESFYDWFERQ	$6.3*10^{-7}$	0
S401	2012	F1	-	1	ESFYDWFER	$>2*10^{-5}$	0
S402	2013	F1	-	1	ESFYDWFE	$>2*10^{-5}$	0
S403	2014	F1	-	1	ESFYDWF	$>2*10^{-5}$	0
S414	2015 and 2016	F1-F1	C-C	1-1	(ESFYDWFERQLGK-Lig')$_2$-14	$3.8*10^{-7}$	++++
S415	2017 and 2018	F1-F1	C-C	1-1	(ESFYDWFERQLGK-Lig')$_2$-23	$1.0*10^{-7}$	++++
S416	2019 and 2020	F1-F1	N-N	1-1	14-(Lig'-ESFYDWFERQLG)$_2$	$9.3*10^{-7}$	+++
S417	2021 and 2022	F1-F1	N-N	1-1	23-(Lig'-ESFYDWFERQLG)$_2$	$9.2*10^{-7}$	+++
S418	2023 and 2024	F1-F1	C-C	1-1	(ESFYDWFERQLGK-Ald)$_2$-17	$1.2*10^{-7}$	++++
S419	2025 and 2026	F6-F6	N-N	2-2	14-(Lig'-EWLDQEWAWVQCEVYGRGCPSEE)$_2$		0
S420	2027 and 2028	F1-F1	N-N	1-1	17-(Ald-ESFYDWFERQLG)$_2$		++
S423	2029 and 2030	F1-F8	C-C	1-3	ESFYDWFERQLG K ACAWPTYWNCG	$6.2*10^{-8}$	0

S425	2031	F1-F6	C-N	1-2	GSLDESFYDWFERQLGKK-Lig'-14- Lig'-EWLDQEWAWVQCEVYGRGCPSEE	$2.4*10^{-9}$	-
S429	2032	F6-F1	C-N	2-1	EWLDQEWAWVQCEVYGRGCPSEE-Lig' -14-Lig'-GSLDESFYDWFERQLGKK	$6.0*10^{-10}$	
S432	2033 and 2034	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K CEVYGRGCPS	$1.8*10^{-7}$	+
S433	2035 and 2036	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K WLDQEWAWVQ	$1.1*10^{-7}$	+
S436	2037 and 2038	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K WLDQEWAWVQCEVYGRGCPS	$5.2*10^{-10}$	+++
S437	2039 and 2040	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K LDQEWAWVQCEVYGRGCPS	$6.9*10^{-10}$	+++
S438	2041 and 2042	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K DQEWAWVQCEVYGRGCPS	$3.0*10^{-8}$	++
S439	2043 and 2044	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K QEWAWVQCEVYGRGCPS	$4.6*10^{-8}$	
S440	2045 and 2046	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K EWAWVQCEVYGRGCPS	$9.9*10^{-8}$	
S441	2047 and 2048	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K WAWVQCEVYGRGCPS	$1.2*10^{-7}$	
S442	2049 and 2050	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K AWVQCEVYGRGCPS	$1.6*10^{-7}$	
S443	2051 and 2052	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K WVQCEVYGRGCPS	$1.7*10^{-7}$	
S444	2053 and 2054	F1-F6	C-C	1-2	ESFYDWFERQLGGGG	$1.9*10^{-7}$	

					K VQCEVYGRGCPS		
S445	2055 and 2056	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K QCEVYGRGCPS	$2.3*10^{-7}$	
S453	2057	F1-F6	C-N	1-2	GSLDESFYDWFERQLGKKK-Ald-17-Ald-KEWLDQEWAWVQCEVYGRGCPSEE	$5.7*10^{-10}$	-
S454	2058 and 2059	F1-F6	C-C	1-2	GSLDESFYDWFERQLGKKK-Ald 17 EWLDQEWAWVQCEVYGRGCPSEEK-Ald	$3.8*10^{-10}$	+++
S455	2060	F6-F1	C-N	2-1	EWLDQEWAWVQCEVYGRGCPSEEK-Ald-18-Ald-GSLDESFYDWFERQLGKK	$1.1*10^{-9}$	++++
S456	2061 and 2062	F1-F6	N-N	1-2	Ald-GSLDESFYDWFERQLGKK 17 Ald-KEWLDQEWAWVQCEVYGRGCPSEE	$2.4*10^{-9}$	+++
S457	2063	F6-F1	C-N	2-1	WLDQEWAWVQCEVYGRGCPSGGSGGSGSLDESFYDWFERQLG	$1.6*10^{-9}$	++++
S458	2064	F6-F1	C-N	2-1	WLDQEWAWVQCEVYGRGCPSGGSGGSGSLDESFYDWFERQLG	$3.2*10^{-9}$	++++
S459	2065	F1-F6	C-N	1-2	GSLDESFYDWFERQLGGGSGGSWLDQEWAWVQCEVYGRGCPS	$7.6*10^{-11}$	-
S467	2066	F6-F1	C-N	2-1	EWLDQEWAWVQCEVYGRGCPSEEK-Ald-16-Ald-GSLDESFYDWFERQLGKK	$6.8*10^{-10}$	++++
S468	2067	F6-F1	C-N	2-1	EWLDQEWAWVQCEVYGRGCPSEEK-Ald-19-Ald-GSLDESFYDWFERQLGKK	$4.0*10^{-10}$	++++

S471	2068	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCPSESFYDWFERQLG	$6.7*10^{-10}$	++++
S481	2069	F6-F1	C-N	2-1	HHHHHHKLDQEWAWVQCEVYGRGCPSESFYDWFERQLG		
S482	2070	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCPSESFYDWFERQLG		
S483	2071	F6-F1	C-N	2-1	LDEWAWVQCVEYGRGCPSESFYDWFERQLG	$5.2*10^{-8}$	0
S484	2072	F6-F1	C-N	2-1	LDQEWAVQCEVYGRGCPSESFYDWFERQLG	$8.7*10^{-8}$	0
S485	2073	F6-F1	C-N	2-1	LDQEWAWVCEVYGRGCPSESFYDWFERQLG	$1.6*10^{-7}$	0
S486	2074	F6-F1	C-N	2-1	LDQEWAWVQCVYGRGCPSESFYDWFERQLG	$5.7*10^{-8}$	0
S487	2075	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRCPSESFYDWFERQLG		
S488	2076	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCSESFYDWFERQLG		
S489	2077	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCPESFYDWFERQLG		
S490	2078	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCESFYDWFERQLG		
S491	2079	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCPSEFYDWFERQLG		
S492	2080	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCPSESFYDWFRQLG		

S493	2081	F6-F1	C-N	2-1	EWLDQEWAWVQCEVYGRGCPSEE-POX-Lys(biotin)		
S494	2082	F6-F1	C-N	2-1	ADQEWAWVQCEVYGRGCPSESFYDWFERQLG	$1.7*10^{-8}$	+
S495	2083	F6-F1	C-N	2-1	LAQEWAWVQCEVYGRGCPSESFYDWFERQL		
S496	2084	F6-F1	C-N	2-1	LDAEWAWVQCEVYGRGCPSESFYDWFERQL		
S497	2085	F6-F1	C-N	2-1	LDQAWAWVQCEVYGRGCPSESFYDWFERQL	$2.5*10^{-9}$	+++
S498	2086	F6-F1	C-N	2-1	LDQEAAWVQCEVYGRGCPSESFYDWFERQL	$5.6*10^{-8}$	+
S499	2087	F6-F1	C-N	2-1	LDQEWAAVQCEVYGRGCPSESFYDWFERQL	$6.2*10^{-10}$	++++
S500	2088	F6-F1	C-N	2-1	LDQEWAWAQCEVYGRGCPSESFYDWFERQL		
S501	2089	F6-F1	C-N	2-1	LDQEWAWVACEVYGRGCPSESFYDWFERQL		
S502	2090	F6-F1	C-N	2-1	LDQEWAWVQCAVYGRGCPSESFYDWFERQL	$3.0*10^{-9}$	+++
S503	2091	F6-F1	C-N	2-1	LDQEWAWVQCEAYGRGCPSESFYDWFERQL		
S504	2092	F6-F1	C-N	2-1	LDQEWAWVQCEVAGRGCPSESFYDWFERQL		
S505	2093	F6-F1	C-N	2-1	LDQEWAWVQCEVYARGCPSESFYDWFERQL		
S506	2094	F6-F1	C-N	2-1	LDQEWAWVQCEVYGAGCPSESFYDWFERQL		
S507	2095	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRACPSESFYDWFERQL		
S508	2096	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCASESFYDWFERQL		
S509	2097	F6-F1	C-N	2-1	LDQEWAWVQSEVYGRGSPSESFYDWFERQL		
S510	2098	F6-F1	C-N	2-1	SLEEEWAQVECEVYGRGCPSGGSGGSGLLDESFYHWFDRQLR	$6.2*10^{-11}$	+++++
S511	2099	F6-F1	C-N	2-1	WLDQEWAWVQCEVYGRGCPSGGSGGSGRVDWLQRNANFYDWFVAELG	$3.8*10^{-9}$	++
S512	2100	F6-F1	C-N	2-1	WLDQEWAWVQCEVYGRGCPSGGSGGSSQAGSAFYAWFDQVLRTV	$2.8*10^{-8}$	++
S513	2101	F6-F1	C-N	2-1	WLDQEWAWVQCEVYGRGCPSGGSGGSQSDAFYSGLWALIGLSDG		
S515	2102	F6	-	2	LDQEWAWVQCEVYGRGCPSPOX-Lys(Biotin)		
S516	2103	F4-F1	C-N	2-1	H-Acy-CLEEwGASL-Tic-QCSGSESFYDWFERQL		
S517	2104	F6-F1	C-N	2-1	SIEEEWAQIKCDVWGRGCPSESFYDWFERQL		
S518	2105	F6-F1	C-N	2-1	RLEEEWAWVQCEVYGRGCPSGSLDESFYDWFERQLG	$1.6*10^{-10}$	+++++
S519	2106	F6-F1	C-N	2-1	SLEEEWAQVECEVYGRGCPSGSLDESFYDWFERQLG	$2.0*10^{-11}$	+++++++
S520	2107	F6-F1	C-N	2-1	SIEEEWAQIKCDVWGRGCPPGLLDESFYHWFDRQLR	$2.0*10^{-11}$	++++++
S521	2108	F4-F1	C-N	2-1	HLCVLEELFWGASLFGYCSGGSLDESFYDWFERQL	$2.7*10^{-8}$	+
S522	2109	F4-F1	C-N	2-1	HLCVLEELFWGASLFGYCSGGRVDWLQRNANFYDWFVAELG		
S523	2110	F6-F10	C-N	2-1	WLDQEWAWVQCEVYGRGCPSDSDWAGYEWFEEQLD	$4.3*10^{-9}$	++
S524	2111	F6-F1	C-N	2-1	HHHHHHKSLEEEWAQVECEVYGRGCPSGSLDESFYDWFERQLG		

7, 9, 12, 13, 14, 17, 19, 20, 21, 22, 23, and 24 represent specific chemical linkers (see Table 3); For FFC: 0 is no effect, + is agonist, - is antagonist. Peptides listed on 3 lines consist of two different peptides, linked N-N or C-C, either by chemical linkage or by being synthesized on the two branches of an amino acid with two amino groups such as, e.g., lysine. Acy = 1-amino-1-cyclohexanecarboxylic acid; Cha = cyclohexylalanine; Aib = 2-aminoisobutyric acid; Hyp = Hydroxyproline; Amino acids which are not capitalized are D-amino acids; Lig = Diaminopropionic acid with a 2-aminohydroxyacetyl group (CO-CH2-O-NH2) on the side chain amino group; Lig' = lysine with a 2-aminohydroxyacetyl group (CO-CH2-O-NH2) on the side chain amino group; Ald = an aldehyde group obtained by periodate oxidation of a serine, either N-terminal or attached to the side chain amino group of lysine.

Results further indicated that S175-S175 dimer peptides (Site 1-Site 1) were less agonistic than S175 monomer peptides (++ vs. +++). S175-S175 dimer peptides having a C-N linkage were less agonistic or equally agonistic as compared to S175-S175 dimer peptides having C-C or N-N linkages. F8-F8 dimer peptides, like the parent monomer, showed no agonist activity.

Example 5: Substrate Phosphorylation Assay (HIR Kinase)

WGA (wheat germ agglutinin)-purified recombinant human insulin receptor was mixed with either insulin or peptide in varying concentrations in substrate phosphorylation buffer (50 mM HEPES (pH 8.0), 3 mM $MnCl_2$, 10 mM $MgCl_2$, 0.05% Triton X-100, 0.1% BSA, 12.5 μM ATP). A synthetic biotinylated substrate peptide (Biotin-KSRGDYMTMQIG) was added to a final concentration of 2 μg/ml. Following a 1 h incubation at RT, the reactions were stopped by the addition of 50 mM EDTA. The reactions were transferred to Streptavidin coated 96-well microtiter plates (NUNC, Cat. No. 236001) and incubated for 1 h at RT. The plates were washed 3 times with TBS (10 mM Tris (pH 8.0), 150 mM NaCl).

Subsequently, a 2000-fold dilution of horseradish peroxidase (HRPO) conjugated phosphotyrosine antibody (Transduction Laboratories, Cat. No. E120H) in TBS was added. The plates were incubated for 30 min and washed 3 times with TBS. TMB (3,3',5,5'-tetramethylbenzidine; Kem-En-Tec, Copenhagen, Denmark) was added. One substrate from Kem-En-Tec was added. After 10-15 min, the reaction was stopped by the addition of 1% acetic acid. The absorbance, representing the extent of substrate phosphorylation, was measured in a spectrophotometer at a wavelength of 450 nM.

The results indicated that the potency of the Site 1-Site 2 dimer, peptide 539, was 0.1 to 1% of that of insulin in all assays tested (Table 8), and the dose-response curves (Figures 17A-17B) had a shape similar to that of insulin dose-response curves, suggesting an insulin-like action

mechanism. In addition, Site 1-Site 2 dimer peptides 537 and 538 were also active as specific insulin receptor antagonists (Table 8; Figures 16A-16C). Notably, Site 2-Site 1 dimer peptide 539 was more active in the kinase assay than Site 1-Site 1 homodimer peptides 521 and 535 (Figures 19A-19B), despite lower FFC potency (Figures 14A-14C; Figures 17A-17B). Similar results are shown in Figures 20A-B and Figures 21A-B. This data suggested that homodimer and heterodimer peptides used different mechanisms of action.

TABLE 8

Pep.	Mon./ Link.	Sequence	SEQ ID NO:	Form	Site IR	HIR K_d (nM)	HIGF-1R K_d (nM)	FFC Pot. (nM)	Kinase Pot. (nM)
HI				na	na				
HIGF-1R				na	na				
521	RP9-6aa-RP9	MADYKDDDDKGSLDESFYDWFERQLGKKGGSGGSGSLDESFYDWFERQLGKKAAA(ETAG)PG	2112	1-1	1-1	25	-	A 3	1400
535	RP9-12aa-RP9	MADYKDDDDKGSLDESFYDWFERQLGKKGGSGGSGGSGGSGSLDESFYDWFERQLGKKAAA(ETAG)PG	2113	1-1	1-1	15	-	A 2	1000
537	RP9-6aa-D8	MADYKDDDDKGSLDESFYDWFERQLGKKGGSGGSWLDQEWAWVQCEVYGRGCPSAAA(ETAG)PG	2114	1-6	1-2	0.092	980	N 10	Inactive
538	RP9-12aa-D8	MADYKDDDDKGSLDESFYDWFERQLGKKGGSGGSGGSGGSWLDQEWAWVQCEVYGRGCPSAAA(ETAG)PG	2115	1-6	1-2	0.080	710	N 10	Inactive
539	D8-6aa-RP9	MADYKDDDDKWLDQEWAWVQCEVYGRGCPSGGSGGSGSLDESFYDWFERQLGKKAAA(ETAG)PG	2116	6-1	2-1	0.530	1500	A 10	110

A = agonist; N = antagonist; na = not applicable; Form. = formula; Mon. = constituent monomers; Link. = linker; Pot. = potency; HI and HIGF-1R are controls; All with tags at both ends; All dimers are linked C-N; Linker sequences are underlined.

Example 6: IR Autophosphorylation Assays

IR activation was assayed by detecting autophosphorylation of an insulin receptor construct transfected into 32D cells (Wang *et al.*, 1993, *Science* **261**:1591-1594; clone 969). The IR transfected 32D cells were

seeded at 5 x 10^6 cells/well in 96-well tissue culture plates and incubated overnight at 37°C. Samples were diluted 1:10 in the stimulation medium (PRIM1640 with 25 nM HEPES pH 7.2) plus or minus insulin. The culture media was decanted from the cell culture plates, and the diluted samples were added to the cells. The plates were incubated at 37°C for 30 min. The stimulation medium was decanted from the plates, and cell lysis buffer (50 mM HEPES pH 7.2, 150 mM NaCl, 0.5% Triton X-100, 1 mM AEBSF, 10 KIU/ml aprotinin, 50 µM leupeptin, and 2 mM sodium orthovanadate) was added. The cells were lysed for 30 min.

In the ELISA portion of the assay, the cell lysates were added to the BSA-blocked anti-IR unit mAb (Upstate Biotechnology, Lake Placid, NY) coated ELISA plates. After a 2 h incubation, the plates were washed 6 times with PBST and biotinylated anti-phosphotyrosine antibody (Upstate Biotechnology) is added. After another 2 h incubation, the plates were again washed 6 times. Streptavidin-Eu was then added, and the plates were incubated for 1 h. After washing the plates again, EG&G Wallac enhancement solution (100 mM acetone-potassium hydrogen pthalate, pH 3.2; 15 mM 2-naphtyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide; 0.1% Triton X-100) was added into each well, and the plates were placed onto a shaker for 20 min at RT. Fluorescence of samples in each well was measured at 615 nm using a VICTOR 1420 Multilabel Counter (EG&G Wallac).

Alternatively, IR autophosphorylation was determined using a holoenzyme phosphorylation assay. In accordance with this assay, 1 µl of purified insulin receptor (isolated from a Wheat Germ Agglutinin Expression System) was incubated with 25 nM insulin, or 10 or 50 µM peptide in 50 µl autophosphorylation buffer (50 mM HEPES pH. 8.0, 150 mM NaCl, 0.025% Triton-X-100, 5 mM $MnCl_2$, 50 µM sodium orthovanadate) containing 10 µM ATP for 45 min at 22°C. The reaction was stopped by adding 50 µl of gel loading buffer containing β-mercaptoethanol (Bio-Rad Laboratories, Inc., Hercules, CA). The samples were run on 4-12% SDS-polyacrylamide gels.

Western Blot analysis was performed by transferring the proteins onto nitrocellulose membrane. The membrane was blocked in PBS containing 3% milk overnight. The membrane was incubated with anti-phosphotyrosine 4G10 HRP labeled antibody (Upstate Biotechnology) for 2 h. Protein bands were visualized using SuperSignal West Dura Extended Duration Substrate Chemiluminescence Detection System (Pierce Chemical Co.).

Example 7: Fluorescence-Based HIR Binding Assays

A. Time-Resolved Fluorescence Resonance Energy Transfer Assays

Time-resolved fluorescence resonance energy transfer assays (TR-FRET) were used for peptide competition studies. In one set of assays, monomer and dimer peptides were tested for the ability to compete with biotinylated RP9 monomer peptide (b-RP9) for binding to HIR-immunoglobulin heavy chain chimera (sIR-Fc; Bass *et al.*, 1996). The assays were performed using a 384-well white microplate (NUNC) with a final volume of 30 µl. Final incubation conditions were in 22 nM b-RP9, 1 nM SA-APC (streptavidin-allophycocyanin), 1 nM Eu^{3+}-sIR-Fc (LANCE™ labeled, PE Wallac, Inc.), 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, and 0.1% BSA (Cohn Fraction V). After 16-24 h of incubation at RT, the fluorescence signal at 665 nm and 620 nm was read on a Victor^2 1420 plate reader (PE Wallac, Inc.). Primary data were background corrected, normalized to buffer controls, and then expressed as percent of specific binding.

Results are shown in Figures 22A-22B. Figure 21A shows b-RP9 competition data. For these figures, the Z'-factor was greater than 0.5 ($Z' = 1-(3\sigma_+ + 3\sigma_-)/|\mu_+ - \mu_-|$; Zhang *et al.*, 1999, *J. Biomol. Screen.* **4**:67-73), and the signal-to-background (S/B) ratio was ~4-5. In Figure 22A, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis of the data according

to the following formula: y = min + (max-min)/(1+10^((logIC_{50}-x)*Hillslope)). This was used to determine IC_{50} values.

In another set of assays, monomer and dimer peptides were tested for the ability to compete with biotinylated-S175 (b-S175) or b-RP9 for binding to sIR-Fc. The TR-FRET assays were performed in a 384-well white microplate with a final volume of 30 µl. Final incubation conditions were in 33 nM b-S175 or 22 nM b-RP9, 1 nM SA-APC, 1 nM Eu^{3+}-sIR-Fc, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, and 0.1% BSA. After 16-24 h of incubation at RT, the fluorescence signal at 665 nm and 620 nm was read on a Victor^2 1420 plate reader. Primary data were background corrected, normalized to buffer controls, and then expressed as % specific binding.

Results are shown in Figures 23A-23B. For these figures, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis of the data, which was used to determine IC_{50} values. Figure 23A shows b-S175 competition data; Figure 23B shows b-RP9 competition data.

B. Fluorescence Polarization Assays

Fluorescence polarization assays (FP) were used for peptide competition studies. In one set of assays monomer and dimer peptides were tested for the ability to compete with fluorescein-RP9 (FITC-RP9) for binding to soluble HIR ectodomain (sIR; Kristensen *et al.*, 1998, *J. Biol. Chem.* **273**:17780-17786). The assays were performed in a 384-well black microplate (NUNC) with a final volume of 30 µl. Final incubation conditions were 1 nM FITC-RP9, 10 nM sIR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.05% BGG (bovine gamma globulin), 0.005% Tween-20®. After 16-24 h of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst™ AD plate reader (LJL BioSystems, Inc.). Primary data were background corrected using 10 nM sIR without FITC-RP9

addition, normalized to buffer controls, and then expressed as percent of specific binding. The Z'-factor was greater than 0.5 and the assay dynamic range was ~125 mP. In Figures 24-27, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis of the data, which was used to determine IC_{50} values. The Z'-factor was greater than 0.5 and the assay dynamic range was ~125 mP. Results are shown in Figures 24A-24B.

In another set of assays, monomer and dimer peptides were tested for the ability to compete with FITC-RP9 for binding to soluble human insulin mini-receptor (mIR; Kristensen *et al.*, 1999, *J. Biol. Chem.* **274**:37351-37356). The FP assays were performed in a 384-well black microplate with a final volume of 30 µl. Final incubation conditions were 2 nM FITC-RP9, 20 nM mIR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.001% BGG, 0.005% Tween-20®. After 16-24 h of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst™ AD plate reader. Primary data were background corrected using 20 nM mIR without FITC-RP9 addition, normalized to buffer controls and then expressed as percent of specific binding. Results are shown in Figures 25A-25B.

Monomers and dimer peptides were also tested for the ability to compete with fluorescein-insulin (FITC-Insulin) for binding to sIR. The FP assays were performed in a 384-well black microplate with a final volume of 30 µl. Final incubation conditions were in 2 nM FITC-Insulin, 20 nM sIR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.05% BGG, 0.005% Tween-20®. After 16-24 h of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst™ AD plate reader. Primary data were background corrected using 20 nM sIR without FITC-Insulin addition, normalized to buffer controls and then expressed as percent of specific binding. Results are shown in Figures 26A-26B.

In other assays, peptide monomers and dimer peptides were tested for the ability to compete with FITC-Insulin for binding to mIR. The FP

assays were performed in a 384-well black microplate with a final volume of 30 µl. Final incubation conditions were 2 nM FITC-Insulin, 20 nM mIR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.05% BGG (bovine gamma globulin), 0.005% Tween-20®. After 16-24 h of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst™ AD plate reader. Primary data were background corrected using 20 nM mIR without FITC-RP9 addition, normalized to buffer controls and then expressed as % specific binding. Results are shown in Figures 27A-27B.

C. Summary

Table 9, below, summarizes the binding data calculated from competition assays using the IR constructs, sIR-Fc, sIR, and mIR, in TR-FRET and FP formats. The data in Table 9 indicate that most dimer peptides (e.g., S291 and S375 or S337), showed greater agonist activity than the corresponding monomer peptides (e.g., H2C or RP9, respectively) in the FFC assay. It was previously demonstrated that an inequality between monomer peptides and insulin was exhibited in competition assays where the assay reporter was a monomer peptide (i.e., RP9 or S175). This inequality was also demonstrated by dimer peptides as seen in Table 9. Table 9 further shows that Group 6 monomer peptides such as E8 (D120) were able to compete with FITC-RP9 or b-RP9 peptides for binding to sIR-Fc, but did not compete peptide ligands, such as FITC-RP9 for binding to mIR. Experiments using different IR constructs thereby allowed differentiation of Site I peptides based on sequence motifs (i.e., Group 6 (Formula 10) *vs.* Group 1 (Formula 1; A6)).

TABLE 9

			TARGET ⇒	sIR-Fc		sIR-Fc		sIR-Fc		sIR		mIR		HIR	
			Label ⇒	b-S175		b-RP9		FITC-RP9		FITC-RP9		FITC-RP9		^{125}I-insulin	
				FRET		FRET		FP		FP		FP		RRA	FFC
Monomer or Dimer	SEQ ID NO:	Link-age	Sequence	IC50 (nM)	Hill	IC50 (nM)	Hill	IC50 (nM)	Hill	IC50 (nM)	Hill	IC50 (nM)	Hill	IC50 (nM)	
H2C	2117		FHENFYDWFVQRVSKK	410	-0.82	1626	-1.03	50	-0.27	37	-0.49	770	-0.89	700	+
S291	1916 and 1917	N-N	(Lig-GGG-H2C)$_2$-9	81	-0.96	250	-0.69			12	-0.35	668	-0.38	1200	++++
RP9	1558		GSLDESFYDWFERQLGKK	6	-0.45	42	-0.69	10	-0.41	0.03	-0.29	49	-0.53	44	+/0
S375	1994	C-N	(RP9-Lig)-14-(RP9-Lig)	7	-0.80	86	-0.67			0.2	-0.22	91	-0.80	200	++++
S337	1960 and 1961	C-C	(RP9-Lig)$_2$-23	0.2	-0.36	14	-0.57	1	-0.37	0.2	-0.28	111	-0.70	11	+++++
S391	2008		truncated-(-GSLDE)RP9(-KK)	59	-0.59	610	-0.56			119	-0.49	284	-0.77	1500	NN
S390	1794		truncated(-GSLD)RP9(-KK)	27	-0.49	127	-0.49			19	-0.64	94	-0.94	620	+
S414	2015 and 2016	C-C	(truncated(-GSLD)RP9(-KK))$_2$-14	92	-0.62	164	-0.73			0.2	-0.25	151	-0.69	NN	NN
S175	1560		GRVDWLQRNANFYDWFVAELG	22	-0.58	64	-0.74	10	-0.56	1	-0.36	167	-1.72	230	+++
S380	2001 and 2002	C-C	(EE-short-S175-Lig)$_2$-9	10	-0.55	23	-0.64			0.5	-0.29	27	-0.49	510	++
E8 (D120)	2118		GGTVWPGYEWLRNA	755	-0.74			207	-0.49			>100000		2200	-
Insulin				59	-0.37	63	-0.46	>100000	-0.25	1250	-	172	-0.78	0.04	+++++

FRET = Time-Resolved Fluorescence Resonance Energy Transfer Assay; FP = Fluorescence Polarization Assay; RRA = Radio-Receptor Assay; FFC = Free Fat Cell Assay; N-N = N-terminal linkage; C-C = C-terminal linkage; All are site 1 (formula 1) monomers or site 1-site 1 (formula 1-formula 1) dimers;

Based on the functional studies outlined above, the following peptide dimers were designed.

SEQ ID NO:	Monom./ Linkers	Sequence
2119	F8-6aa-RP9	HLCVLEELFWGASLFGYCSG**GGSGGS**GSLDESFYDWFERQL
2120	F8-12aa-RP9	HLCVLEELFWGASLFGYCSG**GGSGGSGGSGGS**GSLDESFYDWFERQL
2121	D8-6aa-S175	WLDQEWAWVQCEVYGRGCPS**GGSGGS**GRVDWLQRNANFYDWFVAELG
2122	D8-12aa-S175	WLDQEWAWVQCEVYGRGCPS**GGSGGSGGSGGS**GRVDWLQRNANFYDWFVAELG
2123	F8-6aa-S175	HLCVLEELFWGASLFGYCSG**GGSGGS**GRVDWLQRNANFYDWFVAELG
2124	F8-12aa-S175	HLCVLEELFWGASLFGYCSG**GGSGGSGGSGGS**GRVDWLQRNANFYDWFVAELG
2125	D8-6aa-RP15	HLCVLEELFWGASLFGYCSG**GGSGGS**SQAGSAFYAWFDQVLRTV
2126	D8-6aa-RP6	HLCVLEELFWGASLFGYCSG**GGSGGS**TFYSCLASLLTGTPQPNRGPWERCR
2127	D8-6aa-RP17	HLCVLEELFWGASLFGYCSG**GGSGGS**QSDAFYSGLWALIGLSDG
2128	D8-6aa-Grp 6	HLCVLEELFWGASLFGYCSG**GGSGGS**DSDWAGYEWFEEQLD

Linker sequences are underlined and in bold; Monomer sequences are shown below; All dimers are linked C-N.

SEQ ID NO:	Monomer	Formula	Site	Sequence
1576	F8	4	2	HLCVLEELFWGASLFGYCSG
1558	RP9	1	1	GSLDESFYDWFERQL
2129	D8	6	2	WLDQEWAWVQCEVYGRGCPS
1560	S175	1	1	GRVDWLQRNANFYDWFVAELG
2130	RP15	1	1	SQAGSAFYAWFDQVLRTV
1635	Rp6	2	1	TFYSCLASLLTGTPQPNRGPWERCR
2131	RP17	1	1	QSDAFYSGLWALIGLSDG
1595	Group 6	10	1	DSDWAGYEWFEEQLD

Example 8: Peptide Fusions To The Maltose Binding Protein

A. Cloning

The transfer of interesting peptide sequences from phage display to maltose binding protein (MBP) fusions is desirable for several reasons. First, to obtain a more sensitive affinity estimate, the polyvalency of phage

display peptides should be converted to a monovalent system. For this purpose, the peptide sequences are fused to MBP that generally exists as a monomer with no cysteine residues. Second, competition experiments can be carried out with the same or different peptides, one phage displayed and the other fused to MBP. Lastly, purified peptides can be obtained by cleavage of the fusion protein at a site engineered in the DNA sequence.

Figure 28 shows a schematic drawing of the MBP-peptide construct. In the construct, the N-terminus of the peptide sequence is fused to the C-terminus of the MBP. Two peptide-flanking epitope tags are included, a shortened-FLAG® at the N-terminus and E-Tag at the C-terminus. The corresponding gene fusion was generated by ligating a vector fragment encoding the MBP in frame with a PCR product encoding the peptide of interest. The vector fragment was obtained by digesting the plasmid pMAL-c2 (New England Biolabs) with *Eco*RI and *Hind*III and then treating the fragment with shrimp alkaline phosphatase (SAP; Amersham). The digested DNA fragment was resolved on a 1% agarose gel, excised, and purified by QIAEXII (QIAGEN). The 20-amino acid peptide sequences of interest were initially encoded in the phage display vector pCANTAB5E (Pharmacia). To obtain these sequences, primers were synthesized which anneal to sequences encoding the shortened FLAG® or E-Tag epitopes and also contain the required restriction enzyme sites *Eco*RI and *Hind*III. PCR products were obtained from individual phage clones and digested with restriction enzymes to yield the insert fragment. The vector and insert were ligated overnight at 15°C. The ligation product was purified using QIAquick spin columns (QIAGEN) and electroporations were performed at 1500 v in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 10 ng of DNA and 40 µl of *E. coli* strain ER2508 (RR1 *lon:mini*Tn*10*(Tet^r) (*malB*) (*argF-lac*)*U169* Pro$^+$ *zjc*::Tn*5*(Kan^r) *fhuA2*) electrocompetent cells (New England Biolabs). Immediately after the pulse, 1 ml of pre-warmed (40°C) 2xYT medium containing 2% glucose (2xYT-G) was added and the transformants were grown at 37°C for 1 h. Cell transformants were plated

onto 2xYT-AG plates and grown overnight at 37°C. Sequencing confirmed the clones contained the correct constructs.

B. Small-Scale Expression of Soluble MBP-Peptide Fusion Proteins

E. coli ER2508 (New England Biolabs) carrying the plasmids encoding MBP-peptide fusion proteins were grown in 2xYT-AG at 37°C overnight (250 rpm). The following day the cultures were used to inoculate media (2x YT containing-G) to achieve an OD_{600} of 0.1. When the cultures reached an OD_{600} of 0.6, expression was induced by the addition of IPTG to a final concentration of 0.3 mM and then cells were grown for 3 h. The cells were pelleted by centrifugation and samples from total cells were analyzed by SDS-PAGE electrophoresis. The production of the correct molecular weight fusion proteins was confirmed by Western blot analysis using the monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia).

C. Large-Scale Expression of Soluble MBP-Peptide Fusion Proteins

E. coli ER2508 carrying plasmids encoding the MBP-peptide fusion proteins were grown in 2xYT-AG media for 8 h (250 rpm, 37°C). The cultures were subcultured in 2xYT-AG to achieve an OD_{600} of 0.1 and grown at 30°C overnight. This culture was used to inoculate a fermentor with medium of following composition (g/l): glucose (3.00); $(NH_4)_2SO_4$ 5.00; $MgSO_4 \cdot 7H_2O$ (0.25); KH_2PO_4 (3.00); citric acid (3.00); peptone (10.00); and yeast extract (5.00); pH 6.8.

The culture was grown at 700 rpm, 37°C until the glucose from the medium was consumed (OD_{600} = ~6.0 – 7.0). Expression of the fusion protein was induced by the addition of 0.3 mM IPTG and the culture was grown for 2 h in fed-batch mode fermentation with feeding by 50% glucose at a constant rate of 2 g/l/h. The cells were removed from the medium by centrifugation. Samples of the cell pellet were analyzed by SDS-PAGE

followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product.

D. Purification

The cell pellets were disrupted mechanically by sonication or chemically by treatment with the mild detergent Triton X-100. After removal of cell debris by centrifugation, the soluble proteins were prepared for chromatographic purification by dilution or dialysis into the appropriate starting buffer. The MBP fusions were initially purified either by amylose affinity chromatography or by anion exchange chromatography. Final purification was performed using anti-E-Tag antibody affinity columns (Pharmacia). The affinity resin was equilibrated in TBS (0.025 M Tris-buffered saline, pH 7.4) and the bound protein was eluted with Elution buffer (100 mM glycine, pH 3.0). The purified proteins were analyzed for purity and integrity by SDS-PAGE and Western blot analysis according to standard protocols.

For MBP fusions, IR agonist activity was observed for the Site 1-Site 1 dimer peptides shown in Table 10, below. Additional binding data for the MBP fusions are shown in Table 11, also below.

TABLE 10

Fus.	Monomer/ Linker	Sequence	SEQ ID NO:	Form.	Act.	Site IR	Fus. Conc.	MW (kDa)	K_d(HIR)
426	D8	MBP...NNNNLGIEGRISEFIEGR AQPAMA WLDQEWAWVQCEVYGRGCPSAAA (ETAG)AA	2132	6	N	2	0.76	52.2	1.4×10^{-6}
429	D8-6aa-D8	MBP...NNNNLGIEGRISEFIEGRAQPAMAWLDQEWAWVQCEVYGRGCPSGGSGGS KWLDQEWAWVQCEVYGRGCPSAAA(ETAG)AA	2133	6-6	N-N	2-2	3.2	55.3	1.3×10^{-6}
430	H2C-4aa-RB6	MBP...NNNNLGIEGRISEFIEGRDYKDDDDKFHENFYDWFVRQVSGSGSLDALDRLM RYFEERPSLETAG	2134	1-6	A-	1-1	0.17	54.5	2.1×10^{-6}
431	H2C-6aa-F8	MBP...NNNNLGIEGRISEFIEGRDYKDDDKFHENFYDWFVRQVSGGSGGSHLCVLEE LFWGASLFGYCSGAAA(ETAG)AA	2135	1-4	A-N	1-2	3.3	54.8	4.7×10^{-8}
432	H2C-12aa-F8	MBP-NNNNLGIEGRISEFIEGRDYKDDDKFHENFYDWFVRQVSGGSGGSGGSGGS HLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2136	1-4	A-N	1-2	2.9	55.5	3.5×10^{-8}
433	H2C-9aa-F8	MBP...NNNNLGIEGRISEFIEGRDYKDDDKFHENFYDWFVRQVSGGSGGSGGSHLC VLEELFWGASLFGYCSGAAA(ETAG)AA	2137	1-4	A-N	1-2	2.8	55.2	2.1×10^{-8}
434	G3-12aa-G3	MBP...NNNNLGIEGRISEFIEVRAQPAMARGGGTFYEWFESALRKHGAGGGSGGSG GSGGSRGGGTFYEWFESALRKHGAGAAA(ETAG)AA	2138	1-1	N-N	1-1	0.01	56	3.2×10^{-6}
436	H2C-9aa-H2C	MBP...NNNNLGIEGRISEFIEGRAQPAMAFHENFYDWFVRQVSGGSGGSGGSFHEN FYDWFVRQVSAAA(ETAG)AA	2139	1-1	A	1-1	1.1	54.2	4.1×10^{-7}
437	H2C	MBP...NNNNLGIEGRISEFIEGRAQPAMA FHENFYDWFVRQVSAAA(ETAG)AA	2140	1	N-N	1	0.3	51.5	8.3×10^{-6}
427	G3-6aa-G3	MBP...NNNNLGIEGRISEFIEGRAQPAMARGGGTFYEWFESTLRKHGAGGGSGGSR GGGTFYEWFESALRKHGAGAAA(ETAG)AA	2141	1-1	N-N	1-1	0.02	55.3	3.3×10^{-6}
435	H2C-3aa-H2C-3aa-H2C	MBP...NNNNLGIEGRISEFIEGRAQPAMAFHENFYDWFVRQVSGGSFHENFYDWFV RQVSGGSFHENFYDWFVRQVSAAA(ETAG)AA	2142	1-1-1	A-A-A	1-1-1	2.1	55.5	2.0×10^{-6}
439	H2C-6aa-H2C	MBP...NNNNLGIEGRISEFIEGRAQPAMAFHENFYDWFVRQVSGGSGGSFHEN FYDWFVRQVS(ETAG)AA	2143	1-1	A-A	1-1	1.4	53.9	5.5×10^{-7}
449	H2C-12aa-H2C	MBP...NNNNLGIEGRISEFIEGRAQPAMAFHENFYDWFVRQVSGGSGGSGGSGGSA QPAMAFHENFYDWFVRQVSAAA(ETAG)AA	2144	1-1		1-1	1.5	51.8	6.2×10^{-7}
452	G3	MBP...NNNNLGIEGRISEFIEGRAQPAMARGGGTFYEWFESALRKHGAGAA A(ETAG)AA	2145	1		1	0.15	48.8	7.8×10^{-7}
463	H2C-3aa-H2C	MBP...NNNNLGIEGRISEFIEGRAQPAMAFHENFYDWFVRQVSGGSFHENFYDWFV	2146	1-1	A-A	1-1	1.8	50.1	9.6×10^{-7}

		RQVSAAA(ETAG)AA							
464	LF-H2C	MBP...NNNNLGIEGRISEFIEGRDYKDDDDK FHENFYDWFVRQVSAA(ETAG)AA	2147	1		1	0.045	48.4	3.9×10^{-8}
446	LF-F8	MBP...NNNNLGIEGRISEFIEGRDYKDDDDKHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2148	1		2	1.9	49.1	7.7×10^{-7}
459	SF-RB6	MBP...NNNNLGIEGRISEFGSADYKDLDALDRLMRYFEERPSLAAA(ETAG)AA	2149	3		1	0.069	48.1	7.7×10^{-8}
MBP*	lacZ	**		na			5.1	50	$> 1 \times 10^{-5}$

*MBP (negative control for the fusions) is fused to a small fragment of beta-galactosidase (lacZ); **MBP-lacZ fusion protein was derived from the plasmid pMal-c2 as purchased form NEB. Fus. = fusion; Act. = activity; Conc. = concentration; N = Antagonist; A = Agonist; LF = Long FLAG® epitope (DYKDDDDK; SEQ ID NO:1777); SF = Short FLAG® epitope (DYKD; SEQ ID NO:1545); na = not applicable; Form. = formula; All dimers are linked C-N; Linker sequences are underlined.

TABLE 11

Fusion	Monomer/ Linker	Sequence	SEQ ID NO:	Form.	Site IR	High conc. tested (μM)	Kd (HIR) μM
431-	H2C-6aa-F8	MBP...NNNNLGIEGRISEFIEGRDYKDDDKFHENFYDWFVRQVSGGSGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2150	1-6	1-2	0.2	0.033
431+	H2C-6aa-F8	DYKDDDKFHENFYDWFVRQVSGGSGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2151	1-6	1-2	0.2	0.0074
432-	H2C-12aa-F8	MBP...NNNNLGIEGRISEFIEGRDYKDDDKFHENFYDWFVRQVSGGSGGSGGSGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2152	1-6	1-2	0.2	0.02
432+	H2C-12aa-F8	DYKDDDKFHENFYDWFVRQVSGGSGGSGGSGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2153	1-6	1-2	0.2	0.0038
433-	H2C-9aa-F8	MBP...NNNNLGIEGRISEFIEGRDYKDDDKFHENFYDWFVRQVSGGSGGSGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2154	1-6	1-2	0.2	0.03
433+	H2C-9aa-F8	DYKDDDK FHENFYDWFVRQVSGGSGGSGGS HLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2155	1-6	1-2	0.2	0.004

The concentrations of these fusions vary depending on the expression quality. There are 2 sets of each fusion: uncleaved (-) and cleaved with factor Xa (+). The fusion proteins are in Tris buffer (20 mM Tris, 200 mM NaCl, 1 mM EDTA, 50 mM maltose, pH 7.5) and the cleaved fusions (+) are in the same Tris buffer (500 μl) + 12 μg Factor Xa. (Source of Factor Xa: New England Biolabs). Conc. = concentration; Form. = formula; All dimers are linked C-N; Linker sequences are underlined.

E. BIAcore Analysis

For BIAcore analysis of fusion protein and synthetic peptide binding to insulin receptor, insulin (50 μg/ml in 10 mM sodium acetate buffer pH 5) was immobilized on the CM5 sensor chip (Flowcell-2) by amine coupling. Flowcell-1 was used for background binding to correct for any non-specific binding. Insulin receptor (450 nM) was injected into the flow cell and the binding of IR to insulin was measured in resonance units (RUs). Receptor bound to insulin gave a reading of 220 RU. The surface was regenerated with 25 mM NaOH. Pre-incubation of receptor with insulin in a tube at RT completely abrogated the response units to 16 RU. Thus, the system was validated for competition studies. Several maltose-binding fusion proteins, peptides, and rVabs were pre-incubated with insulin receptor before injecting over the insulin chip for competition studies. The decrease in binding/resonance units indicates that several MBP-fusion proteins can block the insulin-binding site. The results are shown in Tables 12 and 13. The amino acid sequences referred to in the tables are identified in Figures 8 and 9A-9B, except the 447 and 2A9 sequences, which are shown below.

TABLE 12

BIAcore Results—Fusion Proteins Compete for Binding to IR

	Incubation Mixtures	Result (RUs)	Sequence Type
Controls	Insulin Receptor (IR) 450 nM	220	Positive Control
	Insulin (8.7 μM)	16	Negative Control
MBP Fus. Prots.	A7 (20A4)-MBP (4.1 μM) + IR	43	Formula 6 Motif
	D8-MBP (1.6 μM) + IR	56	Formula 6 Motif
	D10-MBP (3.4 μM) + IR	81	Formula 11 Motif
	447-MBP (11.5 μM) + IR	195	hGH Pept. Fus.
	MBP (13 μM) + IR	209	Negative Control

The A7 (20A4), D8, and D10 peptide sequence are shown in Figures 8 and 9A-9B. The 447 peptide sequence is: LCQRLGVGWPGWLSGWCA (SEQ ID NO:2156).

TABLE 13

BIAcore Results -- Synthetic peptides compete for binding to IR

Incubation Mix	% Binding	Result (RUs)	Sequence Type
IR	100	128	Positive control
IR + 20D1	41	51.8	Formula 1 Motif
IR +D8	33	41.6	Formula 6 Motif
IR + 20C11	38	49	Formula 2 Motif (bkg high)
IR + H2	27	34.6	IGF (phosphorylated band)
IR + 2A9	100	128	IGF(bkg high)
IR + 20A4	33	41.8	Formula 6 Motif
IR + p53wt	97	124.5	P53 wild type

The concentration of each peptide was about 40 μM and the concentration of IR was 450 nM. The 20D1, 20A4, and D8 peptide sequences are shown in Figures 8 and 9A-9B. The remaining peptide sequences are as follows: 447 = LCQRLGVGWPGWLSGWCA (SEQ ID NO:2156); 2A9 = LCQSWGVRIGWLTGLCP (SEQ ID NO:2157); 20C11 = DRAFYNGLRDLVGAVYGAWD (SEQ ID NO:1659); H2 = VTFTSAVFHENFYDWFVRQVS (SEQ ID NO:1784).

Regarding preparation of a Site 1 agonist comprising two D117 (H2C) peptides, a linker of only 3 amino acids (12 Å) provided a ligand of greater affinity for Site 1 of IR than a corresponding ligand prepared with a 9 amino acid (36 Å) linking region (Figure 29).

F. Stimulation of Autophosphorylation of IR by MBP-Fusion Peptides

MBP fusion peptides were prepared as described above, and then assayed for autophosphorylation of a insulin receptor construct transfected into 32D cells (Wang *et al.*, 1993; clone 969) (see Example, above). The results of these experiments shown in Figure 30 indicate that the H2C monomer and H2C-H2C homodimer peptides stimulate autophosphorylation of IR *in vivo*. H2C dimer peptides (Site 1-Site 1) with a 6 amino acid linker (H2C-6aa-H2C) were most active in the autophosphorylation assay. Other active dimer peptides are also shown in Figure 30, particularly H2C-9aa-H2C, H2C-12aa-H2C, H2C-3aa-H2C, and F8.

G. Insulin Receptor Binding Affinity and Fat Cell Potency of MBP-Fusion Peptides

Results of assays to determine binding affinity for insulin receptor and fat cell potency of the MBP-fusion peptides are shown in Table 14, below.

TABLE 14

Peptide	SEQ ID NO:	Formula	Site IR	Sequence	HIR Kd (mol/l)	FFC
RB426	2158	F6	2	MBP...NNNNLGIEGRISEFIEGR AQPAMA WLDQEWAWVQCEVYGRGCPS AAA(ETAG)AA	$1.4*10^{-6}$	
RB429	2159	F6-F6	2-2	MBP...NNNNLGIEGRISEFIEGRAQPAMAWLDQEWAWVQCEVYGRGCPSGGSGGSKWLDQEWAWVQCEVYGRGCPSAAA(ETAG)AA	$1.3*10^{-6}$	
RB505M	2160	F4	2	MBP...NNNNLGIEGRISEFIEGRDYKDDDDK HLCVLEELFWGASLFGYCSGAAA(ETAG)AA		
RB517M	2161	F4-F4	2-2	MBP...NNNNLGIEGRISEFIEGRDYKDDDDK HLCVLEELFWGASLFGYCSGGGSGGS HLCVLEELFWGASLFGYCSGAAA(ETAG)AA		
RB515	2162	F4-F4	2-2	MBP...NNNNLGIEGRISEFIEGRDYKDDDDK HLCVLEELFWGASLFGYCSGGGSGGSGGSGGS HLCVLEELFWGASLFGYCSGAAA(ETAG)AA		
RB510	2163	F4-F4-F4	2-2-2	MBP...NNNNLGIEGRISEFIEGRDYKDDDDK HLCVLEELFWGASLFGYCSGGGSGGS HLCVLEELFWGASLFGYCSGGGSGGS HLCVLEELFWGASLFGYCSGAAA(ETAG)AA		
RB437	2164	F1	1	MBP...NNNNLGIEGRISEFIEGRAQPAMA FHENFYDWFVRQVSAAA(ETAG)AA	$8.3*10^{-6}$	
RB463	2165	F1-F1	1-1	MBP...NNNNLGIEGRISEFIEGRAQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSAAA(ETAG)AA	$9.6*10^{-7}$	
RB439	2166	F1-F1	1-1	MBP...NNNNLGIEGRISEFIEGRAQPAMA FHENFYDWFVRQVSGGSGGSFHENFYDWFVRQVS-ETAG	$5.5*10^{-7}$	
RB436	2167	F1-F1	1-1	MBP...NNNNLGIEGRISEFIEGRAQPAMAFHENFYDWFVRQVSGGSGGSGGSFHENFYDWFVRQVSAAA(ETAG)AA	$4.1*10^{-7}$	
RB449	2168	F1-F1	1-1	MBP...NNNNLGIEGRISEFIEGR AQPAMAFHENFYDWFVRQVSGGSGGSGGSGGS AQPAMAFHENFYDWFVRQVSAAA(ETAG)AA	$6.2*10^{-7}$	
RB435	2169	F1-F1-F1	1-1-1	MBP...NNNNLGIEGRISEFIEGRAQPAMAFHENFYDWFVRQVSGGSFHENFYDWFVRQVSGGSFHENFYDWFVRQVSAAA(ETAG)AA	$2.0*10^{-6}$	
RB502	2170	F1	1	MBP...NNNNLGIEGRISEFIEGRDYKDDDDK VRVDWLQRNANFYDWFVAELVAAA(ETAG)AA		
RB508M	2171	F1-F1	1-1	MBP...NNNNLGIEGRISEFIEGRDYKDDDDKVRVDWLQRNANFYDWFVAELGGGSGGSGRVDWLQRNANFYDWFVAELGAAA(ETAG)AA		
RB509M	2172	F1-F1	1-1	MBP...NNNNLGIEGRISEFIEGRDYKDDDDKVRVDWLQRNANFYAWFVAELGGGSGGSGGSGGSGRVDWLQRNANFYDWFVAELGAAA(ETAG)AA		
RB452	2173	F1	1	MBP...NNNNLGIEGRISEFIEGRAQPAMARGGGTFYEWFESALRKHGAGAAA(ETAG)AA	$7.8*10^{-7}$	
RB427	2174	F1-F1	1-1	MBP...NNNNLGIEGRISEFIEGRAQPAMARGGGTFYEWFESTLRKHGAGGGSGGSRGGGTFYEWFESALRKHGAGAAA(ETAG)AA	$3.3*10^{-6}$	
RB434	2175	F1-F1	1-1	MBP...NNNNLGIEGRISEFIEVRAQPAMA RGGGTFYEWFESALRKHGAGGGSGGSGGSGGSRGGGTFYEWFESALRKHGAGAAA(ETAG)AA	$3.2*10^{-6}$	

RB513	2176	F1	1	MBP ..NNNNLGIEGRISEFIEGRDYKDDDDKGSLDESFYDWFERQLGKKAA(ETAG)AA		
RB516	2177	F1-F1	1-1	MBP...NNNNLGIEGRISEFIEGRDYKDDDDKGSLDESFYDWFERQLGKKGGSGGGSGGSLDESFYDWFERQLGKKAAA(ETAG)AA		
RB512	2178	F1-F1	1-1	MBP ...NNNNLGIEGRISEFIEGRDYKDDDDKGSLDESFYDWFERQLGKKGGSGGSGGSGGSGGSLDESFYDWFERQLGKKAAA(ETAG)AA		
RB464	2179	F1	1	MBP ...NNNNLGIEGRISEFIEGRDYKDDDDK FHENFYDWFVRQVSAA(ETAG)AA	$3.8*10^{-18}$	
RB446	2180	F4	2	MBP ...NNNNLGIEGRISEFIEGRDYKDDDDKHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	$7.7*10^{-7}$	
RB459	2181	F3	1	MBP ...NNNNLGIEGRISEFGSADYKDLDALDRLMRYFEERPSLAAA(ETAG)AA	$7.7*10^{-8}$	
RB430	2182	F1-F3	1-1	MBP....NNNNLGIEGRISEFIEGRDYKDDDDKFHENFYDWFVRQVSGGSGGS LDALDRLMRYFEERPSLETAG	$2.1*10^{-6}$	-
RB430	2183	F1-F3	1-1	cleaved DYKDDDKFHENFYDWFVRQVSGGSGSLDALDRLMRYFEERPSLAAA(ETAG)AA	$\sim 4*10^{-9}$	
RB431	2184	F1-F4	1-2	MBP....NNNNLGIEGRISEFIEGRDYKDDDKFHENFYDWFVRQVSGGSGGSGGS HLCVLEELFWGASLFGYCSGAAA(ETAG)AA	$4.7*10^{-8}$	-
RB431	2185	F1-F4	1-2	cleaved DYKDDDKFHENFYDWFVRQVSGGSGGSGHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	$\sim 8*10^{-9}$	
RB432	2186	F1-F4	1-2	MBP-NNNNLGIEGRISEFIEGRDYKDDDKFHENFYDWFVRQVSGGSGGSGGSGGS HLCVLEELFWGASLFGYCSGAAA(ETAG)AA	$3.5*10^{-8}$	-
RB432	2187	F1-F4	1-2	cleaved DYKDDDKFHENFYDWFVRQVSGGSGGSGGSGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	$\sim 6*10^{-9}$	
RB433	2188	F1-F4	1-2	MBP....NNNNLGIEGRISEFIEGRDYKDDDK FHENFYDWFVRQVSGGSGGSGGS HLCVLEELFWGASLFGYCSGAAA(ETAG)AA	$2.1*10^{-8}$	
RB508	2189	F1-F1	1-1	DYKDDDDKVRVDWLQRNANFYDWFVAELGGGSGGSGRVDWLQRNANFYDWFVAELGAAAGAPVPYPDPLEPRSA	$1.5*10^{-7}$	++
RB509	2190	F1-F1	1-1	DYKDDDDKVRVDWLQRNANFYAWFVAELGGGSGGSGGSGGSGRVDWLQRNANFYDWFVAELGAAAGAPVPYPDPLEPRAA	$5.5*10^{-8}$	++
RB505	2191	F4	2	DYKDDDDKHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	$4.8*10^{-7}$	-
RB517	2192	F4-F4	2-2	DYKDDDDKHLCVLEELFWGASLFGYCSGGSGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	$6.0*10^{-6}$	-
RB521	2193	F1-F1	1-1	MADYKDDDDKGSLDESFYDWFERQLGKKGGSGGSGGSLDESFYDWFERQLGKKAAA(ETAG)PG	$4.4*10^{-8}$	+++++
RB535	2194	F1-F1	1-1	MADYKDDDDKGSLDESFYDWFERQLGKKGGSGGSGGSGGSLDESFYDWFERQLGKKAAA(ETAG)PG	$\sim 1.0*10^{-7}$	+++++
RB540	2195	F6	2	MADYKDDDDKWLDQEWAWVQCEVYGRGCPSAAA(ETAG)PG	$\sim 1.0*10^{-7}$	
RB539	2196	F6-F1	2-1	MADYKDDDDKWLDQEWAWVQCEVYGRGCPSGGSGGSGGSLDESFYDWFERQLGKKAAA(ETAG)PG	$7*10^{-10}$	++++
RB537	2197	F1-F6	1-2	MADYKDDDDKGSLDESFYDWFERQLGKKGGSGGSWLDQEWAWVQCEVYGRGCPSAAA(ETAG)PG	$5.9*10^{-11}$	-
RB538	2198	F1-F6	1-2	MADYKDDDDKGSLDESFYDWFERQLGKKGGSGGSGGSGGSWLDQEWAWVQCEVYGRGCPSAAA(ETAG)PG	$1.7*10^{-11}$	-
RB626	2199	F6-F1	2-1	MADYKDEIEAEWGRVRCLVYGRCVGGGGSGGSGGSGGSGGSLDESFYDWFERQLGKKAAA(ETAG)PG	$3.0*10^{-10}$	+++

RB625	2200	F6-F1	2-1	MADYKDDDDKWLDQEWAWVQCEVYGRGCPSQPPPPDITTHRADPQGSLDESFYDWFERQLGKKAAA(ETAG)PG	$3.8*10^{-10}$	+++++
RB622	2201	F6-F1	2-1	MADYKDDDDKWLDQEWAWVQCEVYGRGCPSTPKPPTPPPLSADGSLDESFYDWFERQLGKKAAA(ETAG)PG	$1.0*10^{-9}$	++++
RB596	2202	F1	1	MQNDDGSLDESFYDWFERQLGHHHHHHPG	$9.4*10^{-8}$	
RB569	2203	F1	1	MGSLDESFYDWFERQLGEEEGGDHHHHHHPG	$2.1*10^{-7}$	
RB570	2204	F1	1	MQNDDGSLDESFYDWFERQLGEEEGGDHHHHHHPG	$2.5*10^{-8}$	

ETAG = GAPVPYPDDPLEPR(SEQ ID NO:2205); MBP...NNNNL = fusion junction to MBP at c-terminus of MBP; All dimers are linked C-N.

Example 9: *In Vivo* Assays for Insulin Agonists

To test the *in vivo* activity of dimer peptide S519, an intravenous blood glucose test was carried out on Wistar rats. Male Mol:Wistar rats, weighing about 300 g, were divided into two groups. A 10 µl sample of blood was taken from the tail vein for determination of blood glucose concentration. The rats were anaesthetized with Hypnorm/Dormicum at t = -30 min and blood glucose was measured again at t = -20 min and at t = 0 min. After the t = 0 sample was taken, the rats were injected into the tail vein with vehicle or test substance in an isotonic aqueous buffer at a concentration corresponding to a 1 ml/kg volume of injection. Blood glucose was measured at times 10, 20, 30, 40, 60, 80, 120, and 180 min. The Hypnorm/Dormicum administration was repeated at 20 min intervals. Results shown in Figure 33 demonstrate that the S519 (at 20 nmol/kg) peptide lowered blood glucose levels similar to levels observed for human insulin (at 2.5 nmol/kg) (n=8). The S519 peptide and human insulin showed comparable *in vivo* effects, both in magnitude and onset of response (Figure 33).

Example 10: IGF-1 Binding Peptides

Three major groups of peptide IGF-1-binding peptides were obtained from IGF-1R panning experiments: Site 1 A6 (FyxWF) (SEQ ID NO:1596); Site 1 B6 (FyxxLxxL) (SEQ ID NO:1732), and Site 2 (cysteine loops). See Beasley *et al.* International Application PCT/US00/08528, filed March 29, 2000, and Beasley *et al.*, U.S. Application Serial No. 09/538,038, filed March 29, 2000. Active peptides included 20E2 and RP6 (B6-like; Formula 2), S175 (A6-like; Formula 1), G33 (A6-like; Formula 1), RP9 (A6-like; Formula 1), D815 (Site 2), and D8B12 (Site 2) peptides. The IGF-1 binding peptides were analyzed by various assays, described as follows.

A. Phage Competition

Phage competition studies were performed with Site 1 (RP9) and Site 2 (D815) monomer peptides. Plates were coated with IGF-1R (100 ng/well in carbonate buffer, pH 9.6) overnight at 4°C. Wells were blocked with 4% non-fat milk in PBS for 60 min at room temperature. One hundred microliters of rescued phage were added to each well. Peptides in varying concentrations were added and the mixtures were incubated for 2 h at room temperature. Plates were washed three times with PBS and 100 µl of anti-M13 antibody conjugated to horseradish peroxidase was added to each well. The labeled antibody was incubated at room temperature for 60 min. After washing, 100 µl of ABTS was added per well and the plates read in a microtiter reader at 450 nM.

Phage included RP9 (A6-like; Formula 1); RP6 (B6-like; Formula 2); D8B12 (Site 2); and D815 (Site 2). Peptides included RP9 and D815.

Peptide	Formula	Site IGF-1R	Sequence	SEQ ID NO:
D8B12	6	2	WLEQERAWIWCEIQGSGCRA	1884
D815	6	2	WLDQERAWLWCEISGRGCLS	2206
RP6	2	1	TFYSCLASLLTGTPQPNRGPWERCR	1635
RP9	1	1	GSLDESFYDWFERQLG	1559

Results shown in Figures 34A-34E demonstrate that that RP9 and D815 peptides competed both Site 1 and Site 2 phage. These results illustrate the allosteric nature of the interaction with IGF-1R.

Phage competition studies were also performed with Site 2-Site 1 dimer peptides containing 6- or 12-amino acid linkers. Plates were coated with IGF-1R (100 ng/well in carbonate buffer, pH 9.6) overnight at 4°C. Wells were blocked with 4% non-fat milk in PBS for 60 min at room temperature. One hundred microliters of rescued phage were added to

each well. Peptides in varying concentrations were added and the mixture incubated for 2 h at room temperature. Plates were washed three times with PBS and 100 µl of anti-M13 antibody conjugated to horseradish peroxidase was added to each well. The labeled antibody was incubated for 60 min at room temperature. After washing, 100 µl of ABTS was added per well and the plates read in a microtiter reader at 450 nM. Phage included RP9, RP6, D8B12, and D815. Peptides included D815-6L-RP9 and D815-12L-RP9. Linker sequences are underlined and shown below.

Peptide	Formula	Site IGF-1R	Sequence	SEQ ID NO:
D815-6L-RP9	6-1	2-1	LDQERAWLWCEISGRGCLSGGSGGSGSLDESFYDWFERQLGKK	2207
D815-12L-RP9	6-1	2-1	WLDQERAWLWCEISGRGCLSGGSGGSGGSGGSGSLDESFYDW FERQLGKK	2208

D8B12, D815, RP6, and RP9 amino acid sequences are shown in the previous section. Results shown in Figures 35A-35E demonstrate that dimers competed both Site 1 and Site 2 phage. This indicates that both dimer units were active at IGF-1R.

B. IGF-1 Proliferation Assays

FDC-P2 cells expressing the IL-3 and human IGF-1R receptors were grown in RPMIk-1640 medium supplemented with 15% fetal bovine serum (FBS) and 5% WEHI conditioned medium (containing IL-3) in accordance with routine methods. Prior to an experiment, the cells were pelleted and washed two times in PBS. Following this, cells were resuspended in RPMI-1640 medium with 2% FBS and added to a 96-well plate at a concentration of 2×10^4 cells/well in 75 µl. This was designated as the cell plate.

Peptides were suspended in PPMI-15% FBS (test medium). For the agonist assay, medium was added to rows 2-12 of a 96-well plate. The peptide was added to row 1 in 200 µl of test medium at a final concentration of 60 µM. The peptide was serially diluted (1:1) across rows 2-11. No

peptide was added to row 12 (control; cells without IGF-1). For the antagonist assay, test medium containing 10 ng/ml IGF-1 (ED_{50} test medium) was added to all wells of a 96-well plate. To row 1 was added 100 µl of peptide in ED_{50} test medium at a concentration of 120 µM. The peptide was serially diluted (1:1) across rows 2-11. No peptide was added to row 12 (control; cells with IGF-1).

For both agonist and antagonist assays, 75 µl from the working plates was transferred to the appropriate rows in comparable cell plates. The starting peptide concentration for both agonist and antagonist assays was 30 µM. Each peptide was done in duplicate. Plates were incubated at 37°C for 45-48 h. Ten microliters of WST-1 (Cell Proliferation Reagent, Roche cat # 1 644 807) were added to each well and the plates were read in an ELISA reader (440/700 dual wavelength) each hour for 4 h. Graphs were prepared from the raw data using Sigma Plot. Peptides included:

Peptide	Formula	Site IGF-1R	Sequence	SEQ ID NO:
20E2	2	1	DYKDFYDAIDQLVRGSARAGGTRD	2209
D815	6	2	WLDQERAWLWCEISGRGCLS	2206
G33	1	1	GIISQSCPESFYDWFAGQVSDPWWCW	1600
RP6	2	1	TFYSCLASLLTGTPQPNRGPWERCR	1635
RP9	1	1	GSLDESFYDWFERQLG	1559
S175	1	1	GRVDWLQRNANFYDWFVAELG	1560

Results of the IGF-1 proliferation assays are shown in Figures 36-42. Figure 36 demonstrates that that peptides G33 (Site 1; ED_{50} ~ 10 µM) and D815 (Site 2; ED_{50} ~ 2 µM) showed agonist activity at IGF-1R, whereas peptides RP9 and RP6 showed no agonist activity. Figure 37 demonstrates that that peptides RP6 (Site 1; ED_{50} ~ 1 µM) and RP9 (Site 1; ED_{50} ~ 7 µM) showed antagonist activity at IGF-1R, whereas peptides G33 and D815 showed no antagonist activity. Figure 38 demonstrates that peptides S175

and 20E2 exhibited weak agonist activity at IGF-1R (ED_{50} > 10 μM). Figure 39 shows that D815-RP9 dimers with 6- or 12-amino acid linkers acted as agonists at IGF-1R. Figure 40 shows that dimer peptide D815-6-G33 was inactive as an agonist at IGF-1R. Figure 41 shows that monomer peptide RP6 acted as an antagonist at IGF-1R. The IGF-1 standard curve determined for FDC-P2 cells is shown in Figure 42.

The IGF-1R data for the Site 1 and Site 2 peptides is summarized in Table 15, below.

TABLE 15

Mon./Dimer	Form.	Site IGF-1R	Link.	Sequence	SEQ ID NO:	nM Ki app Kd	nM ED_{50} Growth	Max Action	nM IC_{50} Antag.	Ki/ ED50	Class
IGF-1			NA			0.69	0.30	100	2	2.3	A
rG33	1	1	NA	GIISQSCPESFYDWFAGQVSDPWWCW	1600	1450	500	>50	---	2.9	A
rD815	6	2	NA	WLDQERAWLWCEISGRGCLS	2206	4080	500	>50%	---	8.2	A
RP9	1	1	NA	GSLDESFYDWFERQLG	1559	417	---	<10%	900	0.5	N
D815-G33	6-1	2-1	6 aa	WLDQERAWLWCEISGRGCLSGGSGGSGIISQSCPESFYDWFAGQVSDPWWCW	2210	624	---	<10%	nd		nd
D815-RP9	6-1	2-1	6 aa	WLDQERAWLWCEISGRGCLSGGSGGSGSLDESFYDWFERQLGKK	2211	36	50	>50%	>500	0.8	A
D815-RP9	6-1	2-1	12 aa	WLDQERAWLWCEISGRGCLSGGSGGSGGSGGSGSLDESFYDWFERQLGKK	2212	3	10,000	100	-----	0.0003	A

A = agonists; N = antagonist; nd = not determined; NA = not applicable; Form. = formula; Mon. = monomer; Antag. = antagonism; Link. = linker; Linker sequences are underlined.

Example 11: Panning Peptide Libraries for IGF-1 binding proteins

A. Panning Secondary Libraries

Soluble IGF-1R ("sIGF-1R") was obtained from R&D Systems. The soluble protein (> 95% pure) included the heterotetrameric (alpha 2-beta 2) extracellular domain of IGF-1R isolated from a mouse myeloma cell line. sIGF-1R (500 ng/well) was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, NUNC) and incubated overnight at 4°C. Wells were then blocked with MPBS (PBS buffer pH 7.5 containing 2% Carnation® non-fat dry milk) at room temperature (RT) for 1 h. Eight wells were used for each round of panning for the G33 and RP6 secondary libraries. The phage were incubated with MPBS for 30 min at RT, then 100 µl was added to each well.

For the first round, the input phage titer was 4×10^{13} cfu/ml. For rounds 2 and 3, the input phage titer was approximately 10^{11} cfu/ml. Phage were allowed to bind for 2 to 3 h at RT. The wells were then quickly washed 13 times with 200 µl/well of MPBS. Bound phage were eluted by incubation with 100 µl/well of 20 mM glycine-HCl, pH 2.2 for 30 s. The resulting solution was then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, then plated onto two 24 cm x 24 cm plates containing 2xYT-AG. The plates were incubated at 30° C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80° C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above. A minimum of 72 clones was picked at random from the second, third, and fourth rounds of panning and screened for binding activity. DNA sequencing of the clones determined the amino acid sequences summarized in Figure 43A-43B.

B. Panning Peptide Dimer Libraries

Microtiter plates were coated and blocked by standard methods, as follows. Plates were coated with sIGF-1R (see Example, above) or soluble IR (Bass construct; Bass *et al.,* 1996, *J. Biol. Chem.* **271**:19367-19375) in 0.2 M $NaHCO_3$, pH 9.4. One hundred microliters of solution containing either 50 ng IR or IGF-1R (rounds 1 and 2), 25 ng IR or IGF-1R (round 3), or 12.5 ng IR or IGF-1R (round 4) was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nalge NUNC) and incubated overnight at 4°C. Wells were then blocked with a solution of 2% non-fat milk in PBS (MPBS) at RT for at least 1 h.

Eight wells coated with IR or IGF-1R were used for each round of panning. One hundred microliters of phage were added to each well. For the first round, the input phage titer was 3×10^{13} cfu/ml. For subsequent rounds, the input phage titer was approximately 10^{12} cfu/ml. Phage were incubated for 2-3 h at RT. The wells were then quickly washed 13 times with 300 µl/well of PBS. Bound phage were eluted by incubation with 150 µl/well of 50 mM glycine-HCl, pH 2.0 for 15 min. The resulting solution was pooled and then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, in 2xYT medium for 1 h at 37°C prior to the addition of helper phage, ampicillin, and glucose (2% final concentration).

After incubation for 1 h at 37°C, the cells were spun down and resuspended in 2xYT-AK medium. The cells were then returned to the shaker and incubated overnight at 37°C. Phage amplified overnight were then precipitated and subjected to the next round of panning. A total of 96 clones were picked at random from rounds 3 and 4 and screened for binding activity. Several clones from each pan were further tested for binding to IR or IGF-1R in phage ELISA by competition with soluble peptides as described in Beasley *et al.* International Application PCT/US00/08528, filed March 29, 2000, and Beasley *et al.*, U.S. Application Serial No. 09/538,038,

filed March 29, 2000. Competition was performed by addition of 5 μl of RP9 peptide, recombinant D8 peptide, or both per well, followed by addition of 100 μl of phage per well. Representative peptides are shown in Figures 44A-44B and in Table 16, below.

TABLE 16

Pep.	SEQ ID NO:	Form.	Site IR	Sequence	Description
RP27	2213	6-1	2-1	GLDQEQAWVECEVYGRGCPYGSLDESFYDWFERQLG	No linker
RP28	2214	6-1	2-1	RLEEEWAWVQCEVYGRGCPS**GGSGGS**GSLDESFYDWFERQLG	EEE Stretch in D8
RP29	2215	6-1	2-1	SLDREWACVKCEVYGRGCPC**GGSGGS**GSLDESFYDWFERQLG	Repeat isolate
RP30	2216	6-1	2-1	SLEEEWAQVECEVYGRGCPS**GGSGGS**GSLDESFYDWFERQLG	D8 by design
RP31	2217	6-1	2-1	SLEEEWAQVECEVYGRGCPS**GGSGGS**GLLDESFYHWFDRQLR	D8 & RP9 by design
RP32	2218	6-1	2-1	SIEEEWAQIKCDVWGRGCPP**GGSGGS**GLLDESFYHWFDRQLR	D8 & RP9 by design
RP33	2219	6-1	2-1	QLDLEWAWVQCEVYGRGC**GGS**GSLDESFYDWFERQLG	3 amino acid linker
RP34	2220	6-1	2-1	QLDEEWAGVQCEVYGRGCSLDESFYDWFERQLG	No linker
RP35	2221	6-1	2-1	RLEEEWRWVQCEVYGRGCAA**GGSGGS**GSLDESFYDWFERQLG	EEE Stretch in D8
RP36	2222	6-10	2-1	SLDQEWAWVQCEVYGRGCPS**GGSGGS**DSDWAGYEWFEEQLD	D8 (W1->S)- Group 6 by design

Pep. = peptide; Form. = formula; Linker sequences are shown in bold and underlined; All dimers are linked C-N

C. Determination of Amino Acid Preferences

For both monomer and dimer peptides, amino acid preferences for each peptide were determined as follows. The expected frequency of each of the 20 amino acids at that position was calculated based on codon usage and % doping for that library. This was then compared to the actual frequency of occurrence of each amino acid at every position after four rounds of biopanning. Any amino acid that occurred at a frequency >2-fold was considered preferred. Most preferred amino acid(s) were those that have the greatest fold enrichment after panning. Preferred amino acid sequences for RP9, D8, and Formula 10 (Group 6) peptides are shown below.

TABLE 17

Peptide	Sequence	SEQ ID NO:
RP9	GSLDESFYDWFERQLG	1559
Regular	GLADEDFYEWFERQLR L	2223
w/ Peptide	GQLDEDFYEWFDRQLS A	2224
w/ Insulin	GFMDESFYEWFERQLR W A	2225

Table 17 shows preferred amino acid sequences for RP9 peptides. Residues in bold indicate strong preference; underlined residues indicate positions where more than one amino acid preference is seen. The first column indicates the conditions used for the panning procedure. "RP9" indicates sequence of the parent RP9; "Regular" indicates regular pan as described in methods for panning of random libraries; "w/ peptide" indicates panning in the presence of 2 nM RP9 peptide; "w/ insulin" indicates panning in the presence of 2 nM insulin.

TABLE 18

Peptide	Sequence	SEQ ID NO:
D8 Parent:	WLDQEWAWVQCEVYGRGCPS	2129
Dimer Consensus	sLEE**EW**aQIE**CEVY/WGRGC**ps	2226
Monomer Consensus	sLEE**EW**aQIq**CEIY/WGRGC**ry **W**	1548

Table 18 shows preferred amino acid sequences for D8 peptides. Upper case residues in bold indicate strong preference (>90% frequency); upper case letters, non-bold, indicate some preference (5-15% higher frequency than expected); lower case letters indicate less preference (2-5% higher frequency than expected); similar preferences seen in D8 in both monomer and dimer libraries. The underlined Y/W indicates that both residues are equally preferred at that position. In the original D8 sequence that position is occupied by Y.

TABLE 19

Peptide	Sequence	Type	SEQ ID NO:
Group 6	W(A/E)GYEW(F/L)	preferred core	1549
Group 6	DSDWAGYEWFEEQLD	preferred sequence	1595

Table 19 shows preferred amino acid sequences for Group 6 peptides. Underlined residues indicate preferred N-terminal and C-terminal extensions.

Example 12: Fluorescence-Based hIGF-1R Binding Assays

A. Heterogeneous Time-Resolved Fluorometric Assays

The effect of recombinant peptide G33 (rG33) on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human

IGF-1R (rhIGF-1R) was determined using heterogeneous time-resolved fluorometric assays (TRF; DELFIA®, PE Wallac, Inc.). The rhIGF-1R protein included the extracellular domain of the receptor pre-propeptide, up to amino acid residue 932 (A. Ullrich *et al.*, 1986, *EMBO J.* **5**:2503-2512). Duplicate data points were collected at each concentration of competitor and the lines were designed to represent the best fit to a four-parameter non-linear regression analysis (y = min + (max-min)/(1+10^((logIC_{50}-x)*Hillslope))) of the data, which was used to determine IC_{50} values.

The assay was performed using a 96-well clear microplate (NUNC MaxiSorp) with a final volume of 100 µl. Microtiter plates were coated with 0.1 µg rhIGF-1R in 100 µl of $NaHCO_3$, pH 8.5 buffer, and incubated overnight at room temperature (RT). The plates were washed 3-times with 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl (TBS). This was followed by addition of 200 µl blocking buffer (TBS containing 0.05% Bovine Serum Albumin (BSA, Cohn Fraction V)), and incubated for 1 h at RT. The plates were washed 6-times with a 1 X solution of Wallac's DELFIA® wash concentrate. Competitor was added in a volume of 50 µl and serially diluted across the microtiter plate in TBS containing 0.05% BSA. Non-specific binding (background) was determined in the presence of 60 µM hIGF-1.

Fifty microliters of b-rhIGF-1, 10 nM, diluted in TBS containing 0.05% BSA was added. The plates were incubated for 2 h at RT. After incubation, plates were washed 6-times with a 1X solution of Wallac's DELFIA® wash concentrate. Then the plates were treated with 100 µL of Wallac's DELFIA® Assay Buffer containing a 1:1000 dilution of europium-labeled streptavidin and incubated for 2 h at RT. This was followed by washing 6-times with a 1 X solution of Wallac's DELFIA® wash concentrate. One hundred microliters of Wallac's DELFIA® enhancer was added, and the plates were shaken for 30 min at RT. After shaking, the fluorescence signal at 620 nm was read on a Victor^2 1420 plate reader (PE Wallac, Inc.). Primary data were

background corrected, normalized to buffer controls, and then expressed as % specific binding. The Z'-factor was greater than 0.5 ($Z' = 1-(3\sigma_+ + 3\sigma_-)/|\mu_+ - \mu_-|$; Zhang *et al.*, 1999, *J. Biomol. Screen.* **4**:67-73) and the signal-to-background (S/B) ratio was ~20. The results of these experiments are shown in Figure 45. The IC_{50} value calculated for rG33 is shown in Table 20, below.

The effect of recombinant peptides D815 (rD815), RP9, D815-6aa-G33, D815-6aa-RP9, and D815-12aa-RP9 on the binding of b-rhIGF-1 to rhIGF-1R was determined using the fluorometric assay described above. IGF-1 was used as a control. Duplicate data points were collected at each concentration of competitor and the lines represent the best fit to a four-parameter non-linear regression analysis, which was used to determine IC_{50} values. Results for rD815 are show in Figure 46; results for RP9 are shown in Figure 47; results for D815-6-G33 are shown in Figure 48; results for D815-6-RP9 are shown in Figure 49; and results for D815-12-RP9 are shown in Figure 50; the results for IGF-1 are shown in Figure 51. The IC_{50} values for the rD815, RP9, D815-6aa-G33, D815-6aa-RP9, and D815-12aa-RP9 peptides, and IGF-1 are shown in Table 20, below. Linker sequences are underlined.

TABLE 20

Competitor	Sequence	SEQ ID NO:	IC_{50} (M)
rG33	GIISQSCPESFYDWFAGQVSDPWWCW	1600	1.45×10^{-6} M
rD815	WLDQERAWLWCEISGRGCLS	2206	4.08×10^{-6} M
RP9	GSLDESFYDWFERQLG	1559	4.17×10^{-7} M
D815-6aa-G33	WLDQERAWLWCEISGRGCLSGGSGGSGIIS QSCPESFYDWFAGQVSDPWWCW	2210	6.24×10^{-7} M
D815-6aa-RP9	WLDQERAWLWCEISGRGCLSGGSGGSGSL DESFYDWFERQLGKK	2211	3.57×10^{-8} M
D815-12aa-RP9	WLDQERAWLWCEISGRGCLSGGSGGSGG SGGSGSLDESFYDWFERQLGKK	2212	3.22×10^{-9} M
IGF-1			6.85×10^{-10} M

The order of potency of all peptides or dimers compared to IGF-1 was determined as: IGF-1 > D815-12aa-RP9 >> D815-6aa-RP9 > RP9 ≅ D815-6aa-G33 > rG33 > rD815. These results suggest that the coupling of D815 with RP9 using an extended linker (12 versus 6 amino acids) produced a potent competitor that approximates the affinity of IGF-1 for its own receptor.

B. Time-Resolved Fluorescence Resonance Energy Transfer Assays

The effect of Site 1 peptides, Site 2 peptides, and rhIGF-1 on the dissociation of biotinylated-20E2 (b-20E2, Site 1) from recombinant human IGF-1R was determined using time-resolved fluorescence resonance energy transfer assays (TR-FRET). Best fit non-linear regression analysis of the data, was used to determine dissociation rate constants. Each data point represents a single observation.

The assay was performed using a 96-well white microplate (NUNC) with a final volume of 100 µl. Final incubation conditions were 16.5 nM b-20E2, 2.2 nM SA-APC (streptavidin-allophycocyanin), 2.2 nM Eu^{3+}-rhIGF-1R (LANCE™ labeled, PE Wallac, Inc.), 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, and 0.1% BSA (Cohn Fraction V). Reactions were allowed to reach equilibrium for 6 h at RT. Following this, various peptides or IGF-1 were added at a final concentration of 100 µM or 30 µM, respectively. The addition of peptides or IGF-1 initiated the measurement of dissociation (Time Zero, sec). The fluorescence signal at 665 nm was read on a Victor^2 1420 plate reader (PE Wallac, Inc.) at 30 sec intervals.

Results of these experiments are shown in Figure 52. The buffer controls did not vary over the time interval of study, which demonstrated that the equilibrium was not disturbed by the addition of diluent at Time zero. The addition of excess (> 1000-fold 20E2 K_d for IGF-1R) Site 1 peptides such as H2C, 20E2, or RP6 did not differ depending on specific the peptide used, and the dissociation rates of b-20E2 were similar for these peptides. D8B12 (Site 2 peptide) and IGF-1 (binds both Site 1 and Site 2) did

demonstrate significant differences in the rate of dissociation of b-20E2. This would suggest that these agents act as non-competitive or allosteric regulators of Site 1 binding.

The effect of various peptides or peptide dimers on the binding of biotinylated-20E2 (B-20E2) to recombinant human IGF-1R was determined using TR-FRET assays, described above. For these experiments, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis (y = min + (max-min)/(1+10^((logIC_{50}-x)*Hillslope))) of the data, which was used to determine IC_{50} values.

The assays were performed using a 384-well white microplate (NUNC) with a final volume of 30 µl. Final incubation conditions were 15 nM b-20E2, 2 nM SA-APC, 2 nM Eu^{3+}-rhIGF-1R (LANCE™ labeled, PE Wallac, Inc.), 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, and 0.1% BSA (Cohn Fraction V). After 16-24 h of incubation at RT, the fluorescence signal at 665 nm and 620 nm was read on a Victor^2 1420 plate reader (PE Wallac, Inc.). Primary data were background corrected, normalized to buffer controls, and then expressed as % specific binding. The Z'-factor was greater than 0.5 ($Z' = 1-(3\sigma_+ + 3\sigma_-)/|\mu_+ - \mu_-|$; Zhang *et al.*, 1999, *J. Biomol. Screen.* **4**:67-73) and the signal-to-background (S/B) ratio was ~ 4. Results of these experiments are shown in Figure 53. Table 21, below, shows the IC_{50} values calculated for these experiments. Notably, the C1 peptide showed IGF-1R affinities of ~1 nM (Figure 53) and ~10 nM (Table 21) in these assays.

TABLE 21

Competitor	Sequence	SEQ ID NO:	Formula	Site IGF-1R	IC_{50} (M)
C1	CWARPCGDAANFYDWFVQQAS	1550	1	1	8.80E-10
IGF-1					2.93E-09
RP9	GSLDESFYDWFERQLG	1559	1	1	3.93E-08

20E2	DYKDFYDAIDQLVRGSARAGGTRD	2209	2	1	1.04E-07
E8	GGTVWPGYEWLRNA	2118	10	2	2.53E-07
H2C	FHENFYDWFVQRVSKK	2117	1	1	4.60E-07
S173	LDALDRLMRYFEERPSL	1830	3	1	6.29E-06
D8B12	WLEQERAWIWCEIQGSGCRA	1884	6	2	1.13E-05
A6	SAKNFYDWFVKK	1551	1	1	3.10E-05

C. Fluorescence Polarization Assays

The effect of various peptide monomers and dimers on the binding of fluorescein-RP9 (FITC-RP9) to soluble human insulin receptor-immunoglobulin heavy chain chimera (sIR-Fc; Bass *et al.*, 1996, *J. Biol. Chem.* **271**:19367-19375) was determined using fluorescence polarization assays (FP). For these experiments, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis of the data, which was used to determine IC_{50} values.

The assays were performed in a 384-well black microplate (NUNC) with a final volume of 30 µl. Final incubation conditions were 1 nM FITC-RP9, 10 nM sIR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.05% BGG (bovine gamma globulin), 0.005% Tween-20®. After 16-24 h of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst™ AD plate reader (LJL BioSystems, Inc.). Primary data were background corrected using 10 nM sIR without FITC-RP9 addition, normalized to buffer controls and then expressed as % specific binding. The Z'-factor was greater than 0.5 ($Z' = 1-(3\sigma_+ + 3\sigma_-)/|\mu_+ - \mu_-|$; Zhang *et al.*, 1999, *J. Biomol. Screen.* **4**:67-73) and the assay dynamic range was ~125 mP. In parallel with these experiments, TR-FRET assays were performed using rhIGF-1R and b-20E2, as described above. Results of the FP and TR-FRET experiments are shown in Table 22, below.

TABLE 22

Peptide	FP sIR-Fc	TR-FRET rhIGF-1R	Bndg Ratio IGF-1R / IR	Form.	Site IGF-1R	SEQ ID NO:	Sequence
RP4	17	8100	476	2	1	1552	PPWGARFYDAIEQLVFDNL
S175	10	1650	165	1	1	1560	GRVDWLQRNANFYDWFVA ELG
RP15	28	706	25	1	1	2130	SQAGSAFYAWFDQVLRTV
H2C (D117)	66	600	9	1	1	2117	FHENFYDWFVQRVSKK
20E2 (D118)	51	100	1.9	2	1	2209	DYKDFYDAIDQLVRGSARA GGTRD
RP9	24	33	1.4	1	1	1559	GSLDESFYDWFERQLG
G33	139	178	1.3	1	1	1600	GIISQSCPESFYDWFAGQV SDPWWCW
E8 (D120)	206	175	0.85	10	2	2118	GGTVWPGYEWLRNA
C1 (D112)	52	10	0.19	1	1	1550	CWARPCGDAANFYDWFV QQAS
RP16	6400	961	0.15			1553	VMDARDDPFYHKLSELVT

FP sIR-Fc column shows IC_{50} (nM) values obtained (*vs.* FITC-RP9); TR-FRET rhIGF-1R column shows IC_{50} (nM) values obtained (*vs.* b-20E2); for binding ratio: higher values indicated higher affinity for IR than IGF-1R. Form. = formula; Bndg. = binding.

These results demonstrated that S175, RP4, and RP15 showed high affinities for IR and showed high binding ratios for IGF-1R over IR. H2C, 20E2, RP9, and C1 were slightly less potent than S175, RP4, and RP15 at IR, and these peptides had lower binding ratios for IGF-1R over IR. G33 and E8 were less potent than S175, RP4, and RP15 at IR, and showed comparable binding to IGF-1R and IR. RP16 had poor potency at IR and IGF-1R, but had higher affinity for IGF-1R than IR.

Example 13: IGF-1R Binding Peptides – Additional Isolates

The isolation and characterization of peptides which bind to and subdivide the insulin receptor binding site into multiple, non-overlapping regions designated Site 1 and Site 2 has been previously described (Beasley *et al.*, U.S. Application Serial No. 09/538,038, filed March 29, 2000, published as WO 01/72771; Pillutla *et al.*, U.S. Patent Application Serial No.

09/962,756, filed September 24, 2001; Pillutla *et al.*, 2002, *J. Biol. Chem.* **277**:22590-22594). To identify IGF-1R antagonists, a multi-tiered approach was used. First, Site 1 peptides with greater selectivity for IGF-1R as compared to IR were identified. Second, secondary libraries were generated using information from the primary library pannings. These secondary libraries were designed to define the amino acid requirements for binding, specificity, and affinity.

To determine optimal sequence requirements within the motif, a secondary library based on a clone identified from the random library was made where the flanking regions were held constant, while the core was allowed to change. The library was prepared from doped oligonucleotides so that half of the amino acid residues (on average) in the core sequence were altered per peptide. Panning of these libraries identified substitutions within the core that did or did not affect binding. In an alternative approach, amino acids in the flanking regions conferring binding affinity and/or specificity were defined by designing secondary libraries wherein the core was held constant and the flanking sequences were either doped or randomized. For both types of libraries, amino acids optimal for binding were selected by panning against IGF-1R. Once secondary peptides with the appropriate binding characteristics were identified, a preferred peptide was defined. To do this, the amino acids at each position were optimized based on a comparison of the expected results from the doping strategy and the actual results observed in the binding population.

A. Primary Peptide Libraries

The *E. coli*, strain TG1 (genotype = K12*Δ(lac-pro), supE, thi, hsdΔ5/F'[traD36, proAB, lacI*^{q}*, lacZΔM15*]) was obtained from Pharmacia (Piscataway NJ). DNA fragments coding for peptides containing 40 random amino acids were generated by a PCR-technique using synthetic oligonucleotides. A 145-base oligonucleotide was synthesized to include the sequence $(NNK)_{40}$ where N = A, C, T, or G and K = G or T. This

oligonucleotide was used as the template in PCR reactions along with two shorter oligonucleotide primers, both of which were biotinylated at their 5' ends. The resulting product was purified, concentrated, and ligated to the phagemid pCANTAB5E (Pharmacia). The ligation product was purified and electroporated into competent bacterial cells. The transformants were grown at 37°C for 1 h, pooled and plated onto selection medium. Depending upon the amount of DNA electroporated, the diversity of the random 40mer peptide cell library was found to be between 1.6 X 10^{10} and 1 x 10^{11} independent clones. The phage library was produced by rescue of the cell library according to standard phage preparation protocols (G.P. Smith and J.K. Scott, 1993, *Methods Enzymol.* **217**:228-257). Phage titers were usually at 4 X 10^{13} cfu/ml. In previous experiments, sequencing of randomly selected clones from the cell library indicated that about 54% of all clones were in-frame. The short FLAG sequence (DYKD; SEQ ID NO:1545), was included at the N-terminus as an immunoaffinity tag. In addition, the E-tag epitope (GAPVPYPDPLEPR; SEQ ID NO:XX) was engineered into the carboxy terminus of the peptide. Additional random phage libraries of 20mer peptides were constructed using a similar approach. The diversity of these cell libraries was estimated to be > 1.1 X 10^{11} clones.

B. Secondary and Tertiary Libraries

The desired number of amino acid mutations were introduced in the parental peptide at the codon level when the synthetic DNA template was produced. For example, where a change in 45% of the amino acids was desired (i.e., 9 changes/20 amino acids), then a 60% change at the codon level was needed due to the redundancy of the genetic code (efficiency factor of 0.75). Per position, this translated to 20% doping at the level of DNA synthesis. At the DNA synthesis level, a 20% doping included the following ratio of nucleotides in the synthetic template:

A 80% A, 6.6% C, 6.6% G, 6.6% T

C	6.6% A	80% C,	6.6% G,	6.6% T
G	6.6% A	6.6% C,	80% G,	6.6% T
T	6.6% A	6.6% C,	6.6% G,	80% T

In this chart, the **A, C, T, G** (underlined and in bold) bases represent the original bases in the parental sequence. When the clones from cell libraries were sequenced and the number of amino acid mutations was examined per peptide, the average number of changes was found to correlate to the desired value. After the synthetic template was obtained, the DNA was ligated to the pCANTBA5E phagemid vector to produce the cell library in the TGI strain as previously described. Phage rescue was carried out to produce the phage library used in the panning experiments.

C. Panning of peptide libraries

A standard method was used to coat and block all microtiter plates. Plates were coated with IGF-1R in 0.2 M $NaHCO_3$, pH 9.4. One hundred microliters of solution containing 100 ng of IGF-1R was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nunc) and incubated overnight at 4°C. Wells were then blocked with a solution of 2% non-fat milk in PBS (MPBS) at room temperature (RT) for at least 1 h.

Four to eight wells coated with IGF-1R were used for each round of panning. One hundred microliters of phage were added to each well. For the first round, the input phage titer was ~10^{13} cfu/ml. For subsequent rounds, the input phage titer was approximately 10^{12} cfu/ml. Phage were allowed to bind for 2-3 h at RT. The wells were then quickly washed 13 times with 300 µl/well of PBS. Bound phage were eluted by incubation with 150 µl/well of 50 mM glycine-HCl, pH 2.0 for 5 min. The resulting solution was pooled and then neutralized with Tris-HCl, pH 8.0.

Log phase TG1 cells were infected with the eluted phage, in 2xYT medium for 1 h at 37°C prior to the addition of helper phage, ampicillin and glucose (2% final concentration). After incubation for another hour at 37°C,

the cells were spun down and resuspended in 2xYT-AK medium. The cells were then returned to the shaker and incubated overnight at 37°C. Phage amplified overnight was then precipitated and subjected to the next round of panning. A total of 96 clones were picked at random from rounds 3 and 4 and screened for binding activity.

D. ELISA Analyses of Phage

For phage pools, cells from frozen stocks were grown and phage were prepared as described above. For analysis of individual clones, colonies were picked and phage prepared as described above. Subsequent steps were the same for pooled and clonal phage. Microtiter wells were coated and blocked as described above. Wells were coated with either IGF-1R or IR. Phage resuspended in MPBS (PBS containing 2% non-fat milk) were added to wells (100 μl/well) and incubated at room temperature for 1 h. The phage solution was then removed, and the wells were washed three times with PBS at room temperature.

Anti-M13 antibody conjugated to horseradish peroxidase (Pharmacia Biotech) was diluted 1:3000 in MPBS and added to each well (100 μl/well). Incubation was for another hour at room temperature, followed by PBS washes as described. Color was developed by addition of ABTS solution (100 μl/well; Boehringer). Color development was stopped by adjusting each well to 0.5% SDS. Plates were analyzed at 405 nm using a SpectraMax 340 plate reader (Molecular Devices) and SoftMax Pro software. Data points were averaged after subtraction of appropriate blanks. A clone was considered "positive" if the A_{405} of the well was $\geq$ 2-fold over background.

E. Determination of Amino Acid Preferences

Amino acid preferences for each peptide were determined as follows. The expected frequency of each of the 20 amino acids at that position was

calculated based on codon usage and % doping for that library. This was then compared to the actual frequency of occurrence of each amino acid at every position after four rounds of biopanning. Any amino acid that occurred at a frequency ≥2-fold was considered preferred. The most preferred amino acid(s) were defined as those with the greatest enrichment after panning. Using the amino acid preferences determined for each position, peptides with the most preferred sequences were designed.

Representative monomer and dimer peptides identified by panning secondary libraries for binding to IGF-1R are shown in Figures 54A-54B, 55A-55B, 56A-56B, 57A-57B, 58A-58B, 59A-59B, 60A-60C, 61A-61B, 62A-62B, 63A-63B, and 64A-64B. Primary library pannings produced several peptides, including RP6, RP48, RP52, RP54, RP56, and RP60, described above. Peptides designed according to amino acid preferences (i.e., peptides by design) included RP30-IGF, RP31-IGF, and RP33-IGF.

Example 14: IGF-1 Antagonist Peptides

A. Cells and Reagents

MCF-7 and MiaPaCa cell lines were obtained from the American Type Culture Collection (Manassas, VA). Cells were routinely grown in RPMI1640 medium supplemented with 10% fetal bovine serum and 1% glutamax. The extra-cellular domain of IGF-1R was obtained as a recombinant protein from R&D Systems (Minneapolis, MN).

B. Whole-cell lysates

For qualitative IRS-1 phosphorylation analysis, MCF-7 cells in monolayer cultures (about 80% of confluency) were used. After about 20 h of starvation in serum-free RPMI medium (GibcoBRL), cells were stimulated for 10 min in the same medium containing IGF-1 (Peprotech), or IGF-1 plus peptides (synthetic peptides produced by Research Genetics), or no addition as a negative control. After treatment, cells were rinsed twice with ice-cold PBS containing 0.2 mM PMSF and 1 mM Na_3VO_4 (all from SIGMA).

Cells were scraped into the same buffer and pelleted by centrifugation at 200 x g for 3 min. Lysis was done in RIPA buffer (0.8766% NaCl, 0.11% SDS, 0.5% deoxycholic acid (all from SIGMA), 1% Triton X-100, (Boehringer Mannheim)) containing phosphatase inhibitor cocktails 1 and 2 (SIGMA) and protease cocktail inhibitor tablet (Boehringer Mannheim) for 5 min on ice. Cell lysates were cleared by centrifugation for 5 min at 14 000 x g and the resulting supernatant was snap-frozen in ethanol-dry ice and stored at – 80° C. The protein concentration was determined using the D_C Protein Assay Kit (Bio-Rad Laboratories).

C. Immunoprecipitation and Western Blot Analysis

Immunoprecipitations were performed with pre-cleared lysates for 4 h at 40° C using 0.3-0.5 mg total protein with 1 μg polyclonal anti-IRS-1 antibody (Upstate Biotechnology), and 25 μl protein A/agarose slurry (SIGMA). Agarose beads with immobilized proteins were washed 3 times with IP wash buffer (50 mM Tris pH 7.5 (GibcoBRL), 150 mM NaCl, 1 mM Na_3VO_4, 0.2 mM PMSF). Protein elutions and denaturation were done for 3 min at 95° C in 30 μl of Laemmle sample buffer (Bio-Rad Laboratories) with 0.5 M β-mercaptoethanol (SIGMA).

Immunoprecipitates were subjected to SDS-PAGE on 4-15% Tris-HCl Ready Gels and transferred to Trans-Blot Transfer Medium nitrocellulose membranes (both from Bio-Rad Laboratories). Membranes were blocked with PBS-Tween 20 (SIGMA) containing 2% non-fat milk. For detection of IRS-1 protein, blots were incubated with anti-IRS-1 antibody, followed by secondary antibody goat anti-rabbit IgG, HRP-conjugate. For detection of phosphorylated IRS-1, blots were incubated with monoclonal anti-phosphotyrosine (4G10) HRP-conjugated antibody. All antibodies were obtained from Upstate Biotechnology. Blots were exposed to an enhanced chemifluorescence substrate (ECL Western Blotting Analysis System, Amersham Pharmacia Biotech). Films were developed and fluorescent signal was visualized for qualitative analysis.

D. MCF-7 and MiaPaCa Cell Assays

Peptides produced synthetically were maintained as 30 mM stock in 100% DMSO, while recombinant dimers were diluted in water. All synthetic and recombinant peptides were stored at -80°C. The final concentration of DMSO was < 0.1%. MCF-7 and MiaPaCa (ATCC, Rockville, MD) cells were maintained in RPMI containing 10% FBS. All cells were starved overnight by growing them in RPMI media, which was serum free. Cells were trypsinized, washed twice with PBS before being seeded at 1-3 x 10^3 cells per well in a 96-well plate with a volume of 150 µl/well. All points were done in duplicate in 96-well plates. For antagonist activity assays, immediately before the addition of peptides, all media was gently removed from the wells. Peptides were serially diluted 1:2 in a final volume of 150 µl in a separate plate using RPMI containing 0.1% FBS plus 50 nM IGF-1. This mixture was transferred onto the cells, and the plates were incubated for 72 h at 37°C in a CO_2 incubator. To quantitate cell number, 10 µl of WST-1 reagent (Roche Molecular Biochemicals, Indianapolis, IN) was added to each well and the plates were returned to the 37°C/CO_2 incubator for approximately 2 h. Measurements were then read at 440 nm, with 700 nm used as a reference.

E. Binding (ALPHAScreen) Assays

To assay binding, the relative potencies of peptides as compared to IGF-1 were analyzed in a competition system utilizing biotinylated-human IGF-1 (b-hIGF-1) and His-tagged soluble recombinant human IGF-1R (srhIGF-1R-his; R&D systems, Inc., Minneapolis, MN). Detection of the receptor ligand interaction was measured in an amplified luminescent proximity homogeneous assay (ALPHAScreen; BioSignal-Packard, Montreal). The assay was performed in 384-well Nunc™white polystyrene microplates (Nalge Nunc International, Naperville, IL) with a final volume of 40 µl. Final incubation conditions were 1 nM b-hIGF-1, 10 nM srhIGF-1R-

his, 0.025 M HEPES (pH 7.4 at 25°C), 0.100 M NaCl, 0.1% BSA (Cohn Fraction V; Sigma Chemical Co., St. Louis, MO), 10 μg/ml nickel conjugated acceptor beads, and 10 μg/ml streptavidin conjugated donor beads.

For the first step of the assay, hIGF-1 (PeproTech, Inc., Rocky Hill, NJ), b-hIGF-1 (see below), and peptides were incubated for 2 h at room temperature. Each concentration of competitor was assayed in duplicate. Non-specific binding was determined in the presence of 3×10^{-5} M hIGF-1. In the second step of the assay, acceptor beads were added and the incubation was continued for 0.5 h. In the final step, donor beads were added and the incubation was continued for an additional 1 h. At the end of the incubation period, the fluorescence signal at 520 nm was read on a Fusion-α HT plate reader (Packard BioScience Company, Meriden, CT). Primary data were background corrected, normalized to buffer controls, and then expressed as % specific binding. The data were fit to a four-parameter non-linear regression analysis (y = min + (max-min)/(1+10^((logIC50-x)*Hillslope))), which was used to determine IC_{50} values. The Z'-factor for this assay was greater than 0.7 ($Z' = 1-(3\sigma+ + 3\sigma-)/|\mu+ - \mu-|$) and the signal-to-background (S/B) ratio was between 40 and 70.

Human IGF-1 was biotinylated on free amino groups using Pierce EZ-Link™ Sulfo-NHS-LC-Biotinylation Kit (PN #21430, Pierce, Rockford, IL). Human IGF-1, at 2 mg/ml in PBS, pH 7.2, was incubated at room temperature for 30 min with a 20-fold excess of sulfo-NHS-LC-biotin over theoretical total free amino groups. Unreacted biotins were removed by extensive dialysis (Pierce Slide-A-Lyzer® Dialysis Cassettes) against PBS, and degree of conjugation was determined by HABA (2-(4'-hydroxyazobenzene) benzoic acid) assay (Pierce product literature #21430). Number of biotins per hIGF-1 varied between 3 and 5.

F. FDC-P2 Cell Assays

Peptides produced synthetically were maintained as 30 mM stock in 100% DMSO, while recombinant dimers were diluted in water. All synthetic and recombinant peptides were stored at -80°C. The final concentration of DMSO was < 0.1%. FDC-P2 (obtained from Dr. J. Pierce, National Institutes of Heath, Bethesda, MD) cells were maintained in RPMI containing 15% FBS and 5% WEHI (Genoquest, Germantown, MD) at 37°C in a CO_2 incubator. To initiate experiments, all cells were starved for 5 h in RPMI containing 1% FBS. Cells were seeded at 1 x 10^4 cells per well into 96-well plates at a volume of 75 µl/well. Peptides were added at 2X final concentrations and all points were done in duplicate. For antagonist assays, peptides at 2X concentration were serially diluted 1:2 in a final volume of 75 µl in a separate plate using RPMI containing 0.1% FBS and 1 nM IGF-1. This mixture was transferred onto the cells to yield a final volume of 150 µl. The plates were incubated for 48 h at 37°C in a CO_2 incubator. To quantitate cell number, 10 µl of WST-1 reagent (Roche Molecular Biochemicals, Indianapolis, IN) was added to each well and the plates were returned to the 37°C/CO_2 incubator for approximately 2 h. Measurements were then taken at 440 nm, with 700 nm used as a reference.

G. Results

Peptide RP33-IGF exhibited an affinity for IGF-1R close to that of IGF-1 (9 nM; Table 23). Other peptides, such as RP54 showed affinity in the micromolar range (Table 23). In contrast to the observations made for IR, competition experiments indicated that IGF-1R Site 1 and 2 peptides were able to compete with each other. This suggested that the functional interactions between Site 1 and Site 2 in IGF-1R differed from those found in IR (unpublished data).

To determine if any Site 1 peptides could act as antagonists, proliferation assays were established utilizing IGF-1 and IGF-2 responsive

human tumor cell lines. Sixteen human tumor cell lines were screened for their ability to proliferate in the presence IGF-1 and IGF-2 under serum-free conditions. Two cell lines, MCF-7 (breast carcinoma) and MiaPaCa (pancreatic carcinoma), showed the best dose response curves for IGF-1 (ED_{50} = 5 nM; Figures 65A-65F), and were used for subsequent experiments.

Peptides were synthesized and screened in the proliferation assay at an IGF-1 dose ten times the ED_{50} (50 nM). Several antagonist peptides were identified, including RP33-IGF, which consistently blocked IGF-1 and IGF-2 proliferation of both MCF-7 and MiaPaCa (Figures 66B-66C). In addition, peptides RP52 and RP54 were found to act as antagonists in at least one cell line (Table 26; Figures 70A-70B). Peptides RP52 and RP54 are classified as miscellaneous peptides, which were not categorized into any of the formulae (e.g., Formula 1, Formula 2, etc.) disclosed herein.

Experiments were then performed to determine whether antagonist peptides could block receptor activation at the level of key signaling intermediate, IRS-1. First, the optimal time and concentration of IGF-1 needed for maximal activation of IRS-1 was established (Figures 67A-67B and Figures 68A-68B). Maximum phosphorylation of IRS-1 was observed after 10 min of treatment and was followed by a drop-off of the signal (Figures 67A-67B). This pattern was presumably due to degradation of the IRS-1 protein by a mechanism involving proteasomes (Lee *et al.*, 2000, *Mol. Cell. Biol.*, 2000, **20**:1489-1496). Second, RP33-IGF was compared to two unrelated peptides. The RP33-IGF peptide inhibited IRS-1 phosphorylation, whereas the unrelated peptides had no effect in the proliferation assay (Figures 69A-69B).

The RP6KK peptide was also tested for activity, since the RP33-IGF peptide was originally derived from the RP6KK sequence. Both RP6KK and RP33-IGF were found to effectively block activation of IRS-1 by IGF-1 (Figures 69A-69B). At the concentration used, greater than 90% of the protein was unphosphorylated, indicating that both peptides efficiently

blocked IGF-1R activation. However, RP33-IGF differed from RP6KK by 11 amino acids, and RP33-IGF was a superior IGF-1R antagonist in the cell proliferation assays (Tables 24-25). The difference in biological activity did not appear to be related to stability of the peptides since both were found to remain intact during the course of the assays (unpublished data).

TABLE 23

Pep. (Clone)	Sequence	Site*	Formula	Affin. (μM)	Activ.[&]	IC_{50} (μM)
RP33-IGF	SFYS[C]LESLVNGPAEKSRGQWDG[C]RKK SEQ ID NO:2232	1	2	0.009	Antag.	0.1 - MCF-7 0.7 -MiaPaCa
RP6KK	TFYS[C]LASLLTGTPQPNRGPWER[C]RKK SEQ ID NO:2233	1	2	0.19	Antag.	
RP52 (20C-3-A3-IGFR)	EISFR[C]QLFVLAGMHP[C]PVDVGGEGFE SEQ ID NO:2245		Misc.	ND	Antag.	0.5 - MCF-7
RP54 (20C-4-A7-IGFR)	EGSSI[C]NLLARAQIVELAL[C]EMGVQEE SEQ ID NO:2246		Misc.	1.6	Antag.	2.5 - MCF-7 4.3 - MiaPaCa

Peptide antagonists of IGF-1R identified from primary and secondary library pannings. *site to which a peptide binds was assigned based on competition assays using both IR and IGF-1R as target; [+]affinity was determined using the AlphaScreen assay versus IGF-1 as described herein; [$]antagonism was determined from the proliferation assays in the presence of IGF-1 using MCF-7 and MiaPaCa cells as described herein; ND = not done; Pep. = peptide; Affin. = affinity; Antag. = antagonist; Misc. = miscellaneous peptide; Cysteine residues are boxed.

TABLE 24

IGF-1R Antagonists in MCF-7 Cells

Peptide	Form.	Site IGF-1R	Cellular IC_{50} (M)	Binding IC_{50} (M)	Sequence
H2C-A-H6	1	1	4.0E-07	3.2E-05	VGRASGFPENFYDWFGRQLSLQSGEQ SEQ ID NO:2228
C1KK	1	1	2.8E-06	4.2E-08	DYKDCWARPCGDAANFYDWFVQQASKK SEQ ID NO:2266
RP33K-IGF	2	1	2.1E-08	1.8E-09	SFYSCLESLVNGPAEKSRGQWDGCRK SEQ ID NO:2266
RP6KK	2	1	9.0E-06	1.8E-07	TFYSCLASLLTGTPQPNRGPWERCRKK SEQ ID NO:2233
RP54	Misc.		4.3E-06	4.9E-07	EGSSICNLLARAQIVELALCEMGVQEE SEQ ID NO:2246
RP52	Misc.		4.5E-07	3.0E-05	EISFRCQLFVLAGMHPCPVDVGGEGFE SEQ ID NO:2245
RP30-IGF-12-RP30-IGF	2-2	1-1	3.4E-06	2.4E-07	ADYKDSFYSCLESLVNGGAERSDGQWEGCRGGSGGSGGSGGSSFYSCLESLVNGGAERSDGQWEGCRAAAG APVPYPDPLEPRPG; SEQ ID NO:2241
D8B12-12-RP9	6-1	2-1	6.9E-06	N/A	ADYKDWLEQERAWIWCEKGSGCRAGGSGGSGGSGGSDDDKGSLDESFYDWFERQLGKKAAAGAPVPYPDPLE PRG; SEQ ID NO:2244

Monomer and dimer peptides which block IGF-1 activity in cell proliferation assays in MCF-7 cells. Form. = formula; Misc. = miscellaneous sequence; Cysteine pairs are shaded and underlined; FY, WF, and L residues from Formula 1 and Formula 2 motifs are shaded and shown in bold.

TABLE 25

IGF-1R Antagonists in MiaPaCa Cells

Peptide	Form.	Site IGF-1R	Cellular IC_{50} (M)	Binding IC_{50} (M)	Sequence
RP30-IGF	2	1	2.0E-06	1.6E-07	SFYSCLESLVNGGAERSDGQWEGCR SEQ ID NO:2234
RP43	2	1	1.0E-07	1.9E-07	SFYSCLGSLLTGAPQPIRGAWDRCR SEQ ID NO:2235
RP33K-IGF	2	1	2.0E-07	1.8E-09	SFYSCLESLVNGPAEKSRGQWDGCRK SEQ ID NO:2266
L-RP9ex	1	1	1.3E-05	2.2E-06	ADYKDWLDQERAWLWCEISGRGCLSAAAGAPADYKDDDDKGSLDESFYDWLERQLKKAAAGAPVPYPDPLEPR PG; SEQ ID NO:2231
RP54	Misc.		5.9E-06	4.9E-07	EGSSICNLLARAQIVELALCEMGVQEE SEQ ID NO:2246
RP56	Misc.		9.4E-06	6.0E-05	EGYSWLRDVLMEKQAQLKREGSVGRQE SEQ ID NO:2247
RP30-IGF-12-RP30-IGF	2-2	1-1	4.6E-06	2.4E-07	ADYKDSFYSCLESLVNGGAERSDGQWEGCRGGSGGSGGSGGSSFYSCLESLVNGGAERSDGQWEGCRAAAG APVPYPDPLEPRPG; SEQ ID NO:2241
D8B12-12-RP9	6-1	2-1	4.7E-05	N/A	ADYKDWLEQERAWIWCEKGSGCRAGGSGGSGGSGGSDDDKGSLDESFDWLERQLGKKAAAGAPVPYPDPLE PRG; SEQ ID NO:2241

Monomer and dimer peptides which block IGF-1 activity in cell proliferation assays in MiaPaCa cells. Form. = formula; Misc. = miscellaneous sequence; Cysteine pairs are shaded and underlined; FY, WF, and L residues from Formula 1 and Formula 2 motifs are shaded and shown in bold.

TABLE 26

IGF-1R Antagonists in FDC-P2 cells

Peptide	Form.	Site IGF-1R	Cellular IC_{50} (M)	Binding IC_{50} (M)	Sequence
RP30-IGF	2	1	4.0E-06	1.6E-07	SFYSCLESLVNGGAERSDGQWEGCR SEQ ID NO:2234
RP9-lig	1	1	3.2E-04	2.2E-07	GSLDESFYDWFERQLGKK-Lig SEQ ID NO:
lig-RP9	1	1	8.8E-08	1.5E-06	Lig-GSLDESFYDWFERQLGKK SEQ ID NO:
RP43	2	1	7.0E-06	1.9E-07	SFYSCLGSLLTGAPQPIRGAWDRCR SEQ ID NO:2235
H2C-A-H6	1	1	3.0E-07	3.2E-05	VGRASGFPENFYDWFGRQLSLQSGEQ SEQ ID NO:2228
RP6KK	2	1	1.0E-06	N/A	TFYSCLASLLTGTPQPNRGPWERCRKK SEQ ID NO:2236
C1	1	1	4.0E-06	4.2E-08	DYKDCWARPCGDAANFYDWFVQQAS SEQ ID NO:2230
RP33-IGF	2	1	7.0E-06	1.9E-06	SFYSCLESLVNGPAEKSRGQWDGCR SEQ ID NO:2232
RP6	2	1	5.0E-06	3.5E-07	TFYSCLASLLTGTPQPNRGPWERCR SEQ ID NO:2236
RP9	1	1	2.0E-06	9.7E-07	GSLDESFYDWFERQLGKK SEQ ID NO:2229
RP9-RP9 (C-C)	1-1	1-1	3.0E-05	1.2E-07	$(GSLDESFYDWFERQLGKK)_2$-17 SEQ ID NO:2237
RP9-RP9 (C-N)	1-1	1-1	3.0E-05	1.7E-07	GSLDESFYDWFERQLGKK-Lig)-19-(Lig-GSLDESFYDWFERQLGKK) SEQ ID NO:2238
G33-RP9	1-1	1-1	1.0E-06	N/A	ADYKDGIISQSCPESFYDWFAGQVSDPWWCWGSLDESFYDWFERQLAAAGAPVPYPDPLEPRPG SEQ ID NO:2240

RP9-L-RP9	1-1	1-1	9.0E-07	3.4E-06	ADYKDDDDKGSLDESFYDWFERQLAAAGAPADYKDDDDKGSLDESFYDWFERQLKKAAAGAPVPYPDPL EPRPG; SEQ ID NO:2239
RP9-L-RP6	1-2	1-1	3.0E-06	N/A	ADYKDDDDKGSLDESFYDWFERQLAAAGAPADYKDTFYSCIASLTGTPQPNRGPWERCRAAAGAPVPY PDPLEPRPG; SEQ ID NO:2242
G33-D8B12	1-6	1-2	3.0E-06	N/A	ADYKDGIISQSCPESFYDWFFAGQVSDPWWCWWLEQERAWIWCEKGSGCRAAAAGAPVPYPDPLEPRP G; SEQ ID NO:2243
D8B12-RP9	6-1	2-1	1.0E-05	N/A	ADYKDWLEQERAWIWCEIQGSGCRAGSLDESFYDWFERQLGKKAAAGAPADYKDG SEQ ID NO:2244

Monomer and dimer peptides which block IGF-1 Activity in cell proliferation assays in FDC-P2 cells. Form. = formula; Lig = Diaminopropionic acid with a 2-aminohydroxyacetyl group (CO-CH2-O-NH2) on the side chain amino group; Numbers such as 17, 19, 12, represent specific chemical linkers (see Table 3); C-C = C-terminal to C-terminal linkage; N-N = N-terminal to N-terminal linkage; Cysteine pairs are shaded and underlined; FY, WF, and L residues from Formula 1 and Formula 2 motifs are shaded and shown in bold.

Example 15: IGF-1 Agonist Peptides

A. MCF-7 and MiaPaCa Cell Assays

Peptides produced synthetically were maintained as 30 mM stock in 100% DMSO, while recombinant dimers were diluted in water. All synthetic and recombinant peptides were stored at -80°C. The final concentration of DMSO was < 0.1%. MCF-7 and MiaPaCa (ATCC, Rockville, MD) cells were maintained in RPMI containing 10% FBS. All cells were starved overnight by growing them in serum-free RPMI media. Cells were trypsinized, washed twice with PBS before being seeded at 1-3 x 10^3 cells per well in a 96-well plate in a volume of 150 µl/well. All points were done in duplicate in 96-well plates. For agonist activity assays, immediately before the addition of peptides, all media was gently removed from the wells. Peptides were serially diluted 1:2 in a final volume of 150 µl in a separate plate using RPMI containing 0.1% FBS. The diluted peptide solutions were transferred onto the cells, and the plates were incubated for 72 h at 37°C in a CO_2 incubator. To quantitate cell number, 10 µl of WST-1 reagent (Roche Molecular Biochemicals, Indianapolis, IN) was added to each well and the plates were returned to the 37°C/CO_2 incubator for approximately 2 h. Measurements were then taken at 440 nm, with 700 nm used as a reference.

B. FDC-P2 Cell Assays

Peptides were maintained and stored as indicated above. FDC-P2 cells (obtained from Dr. J. Pierce, NIH) were maintained in RPMI containing 15% FBS and 5% WEHI (Genoquest, Germantown, MD) at 37°C in a CO_2 incubator. To initiate experiments, all cells were starved for 5 h in RPMI containing 1% FBS. Cells were seeded at 1 x 10^4 cells per well into 96-well plates at a volume of 75 µl/well. Peptides were added at 2X final concentration and all points were done in duplicate. For agonist assays, peptides at 2X concentration were serially diluted 1:2 in a final volume of 75

μl in a separate plate using RPMI containing 0.1% FBS. The diluted peptide solutions were transferred onto the cells to yield a final volume of 150 μl. The plates were incubated for 48 h at 37°C in a CO_2 incubator. To quantitate cell number, 10 μl of WST-1 reagent (Roche Molecular Biochemicals, Indianapolis, IN) was added to each well and the plates were returned to the 37°C incubator for approximately 2 h. Measurements were taken at 440 nm, with 700 nm used as a reference.

For these experiments, potencies of peptide competition were determined using the AlphaScreen assay format. Primary data were background corrected, normalized to buffer controls and then expressed as % specific binding. The data were fit to a four-parameter non-linear regression analysis (y = min + (max-min)/(1+10^((logIC_{50}-x)*Hillslope))), which was used to determine IC_{50} values. The Z'-factor for this assay is greater than 0.7 ($Z' = 1-(3\sigma_+ + 3\sigma_-)/|\mu_+ - \mu_-|$) and the signal-to-background (S/B) ratio was between 40 and 70.

C. Results

Several IGF-1R agonist peptides were identified which consistently stimulated proliferation of both MCF-7 and MiaPaCa cells (Tables 27-28; Figures 73A-73D and Figures 74A-74I). Monomer peptides with IGF-1R agonist activity included RP60, RP48, G33, C1, and L-RP9ex (Tables 27-28). Dimer peptides with IGF-1R agonist activity included RP30-IGF-12-D112, RP30-IGF-12-RP31-IGF, RP31-IGF-12-RP30-IGF, D112-12-RP30-IGF, RP6-L-D8B12, D8B12-12-RP9, D112-12-D112, RP9-12-RP9, and RP9-L-RP6 (Tables 27-28). Agonist peptides were also identified using FDC-P2 cell proliferation assays (Table 29). Monomer peptides with IGF-1R agonist activity included G33-lig, G33, S175, D815, lig-D815, RP31-IGF, and D815 (Table 29). Dimer peptides with IGF-1R agonist activity included RP6-RP9, G33-6-G33, and D815-RP9 (Table 29).

In addition, peptides with agonist or antagonist activity in MCF-7 or MiaPaCa cell proliferation assays were shown to compete against IGF-1 for binding to IGF-1R (Figures 71A-71F and Figures 72A-72E). Potencies of peptide competition were determined using the AlphaScreen assay format for peptide monomers RP60, RP48, sG33, L-RP9ex, and 12-RP9ex (Figures 71A-71F). Potencies were also determined for dimer peptides rRP30-IGF-12-D112, rRP30-IGF-12-RP31-IGF, rRP31-IGF-12-RP30-IGF, rD112-12-RP30-IGF, and rD112-12-D112 (Figures 72A-72E).

The biological response of the monomers and dimers in the FDC-P2 (myeloid cells; IGF-1R/IGF-1R receptor), MCF-7 (breast cancer cells; hybrid IGF-1R/IR receptor) and MiaPaCa (pancreatic cancer cells; hybrid IGF-1R/IR receptor) assays were compared (Table 30). In some instances, a modulatory effect (agonism or antagonism) was seen in certain cell lines but not in others. For example, the RP30-IGF peptide exhibited antagonist activity in FDC-P2 and MiaPaCa cells, but not in MCF-7 cells (Table 30). The C1 peptide exhibited antagonist activity in FDC-P2 and MCF-7 cells, but not in MiaPaCa cells. The RP9-RP6, L-RP9ex, and D8B12-12-RP9 peptides exhibited either antagonist or agonist activity depending on the cell line used (Table 30). Therefore, it is possible to use the peptides of the invention to target specific cell types with specific modulatory effects.

TABLE 27

IGF-1R Agonists in MCF-7 Cells

Peptide	Form.	Site IGF-1R	Cellular EC_{50} (M)	Binding IC_{50} (M)	Sequence
G33	1	1	3.0E-07	6.0E-07	GIISQSCPESFYDWFAGQVSDPWWCW SEQ ID NO:2249
12-RP9ex	1	1	1.0E-05	4.5E-07	ISGRGCLSGGSGGSGGSGGSGSLDESFYDWFERQLGKKAAAGAPVPYPDPLEPRPG SEQ ID NO:2250
L-RP9ex	1	1	4.2E-06	2.2E-06	ADYKDWLDQERAWLWCEISGRGCLSAAAGAPADYKDDDDKGSLDESFYDWFERQLKKAAAGAPVPYPDPLEPRP G; SEQ ID NO:2231
RP48	Misc.		3.2E-06	6.6E-07	EDWLRCLGVILSGGLTELANTGCVQGE SEQ ID NO:2261
RP60	Misc.		2.1E-06	1.6E-05	ELQGFCELLATVTGVTGLGCLDYQPIE SEQ ID NO:2262
RP9-12-RP9	1-1	1-1	2.4E-06	N/A	ADYKDDDDKGSLDESFYDWFERQLGKK GGSGGSGGSGGS DDDKGSLDESFYDWFERQLGKK AAAGAPVPYPDPLEPRG; SEQ ID NO:2254
RP9-L-RP6	1-2	1-1	1.3E-06	N/A	ADYKDDDDKGSLDESFYDWFERQLAAAGAPADYKDTFYSCLASLLTGTPQPNRGPWERCRAAAGAPVPYPDPLE PRPG; SEQ ID NO:2242
D112-12-RP30-IGF	1-2	1-1	2.5E-07	1.7E-07	ADYKDCWARPCGDAANFYDWFVQQASKKGGSGGSGGSGGSSFYSCLESLVNGGAERSDGQWEGCRAAAGAP VPYPDPLEPRPG; SEQ ID NO:2257
D112-12-D112	1-1	1-1	3.5E-07	6.2E-07	ADYKDCWARPCGDAANFYDWFVQQASKKGGSGGSGGSGGSCWARPCGDAANFYDWFVQQASKKAAAGAPVP YPDPLEPRPG; SEQ ID NO:2255
RP30-IGF-12-D112	2-1	1-1	4.1E-07	1.1E-06	ADYKDSFYSCLESLVNGGAERSDGQWEGCRGGSGGSGGSGGSCWARPCGDAANFYDWFVQQASKKAAAGAP VPYPDPLEPRPG; SEQ ID NO:2258
RP6-L-D8B12	2-6	1-2	7.1E-07	N/A	ADYKDTFYSCLASLLTGTPQPNRGPWERCRAAAGAPADYKDWLEQERAWIWCEKGSGCRAAAAGAPVPYPDPLE PRPG; SEQ ID NO:2263
RP30-IGF-12-RP31-IGF	2-6	1-2	1.1E-07	1.1E-06	ADYKDSFYSCLESLVNGGAERSDGQWEGCRGGSGGSGGSGGSGVDEVRAWLWCQISGLGCESKKAAAGAPVP YPDPLEPRPG; SEQ ID NO:2264
RP31-IGF-12-RP30-IGF	6-2	2-1	3.0E-06	7.2E-08	ADYKDGVDEVRAWLWCQISGLGCESKKGGSGGSGGSGGSSFYSCLESLVNGGAERSDGQWEGCRAAAGAPVP YPDPLEPRPG; SEQ ID NO:2265
D8B12-12-RP9	6-1	2-1	3.5E-07	N/A	ADYKDWLEQERAWIWCEKGSGCRAGGSGGSGGSGGSDDDKGSLDESFYDWFERQLGKKAAAGAPVPYPDPLE PRG; SEQ ID NO:2241

Monomer and dimer peptides which stimulate cell proliferation using the MCF-7 cells. Form. = formula; N/A = not available; Misc. = miscellaneous sequence ; Cysteine pairs are shaded and underlined; FY, WF, and L residues from Formula 1 and Formula 2 motifs are shaded and shown in bold. .

TABLE 28

IGF-1R Agonists in MiaPaCa Cells

Peptide	Form.	Site IGF-1R	Cellular EC50 (M)	Binding IC50 (M)	Sequence
RP48	Misc.		7.0E-06	6.6E-07	EDWLRCLGVILSGGLTELANTGCVQGE SEQ ID NO:2261
RP60	Misc.		3.1E-06	1.6E-05	ELQGFCELLATVTGVTGLGCLDYQPIE SEQ ID NO:2262
RP9-L-RP6	1-2	1-1	1.0E-06	N/A	ADYKDDDDKGSLDESFYDWFERQLAAAGAPADYKDTFYSCLASLLTGTPQPNRGPWERCRAAAGAPVPYPDPLE PRPG; SEQ ID NO:2242
D112-12-RP30-IGF	1-2	1-1	2.2E-06	1.7E-07	ADYKDCWARPCGDAANFYDWFVQQASKKGGSGGSGGSGGSSFYSCLESLVNGGAERSDGQWEGCRAAAGAP VPYPDPLEPRPG; SEQ ID NO:2257
D112-12-D112	1-1	1-1	2.5E-06	6.2E-07	ADYKDCWARPCGDAANFYDWFVQQASKKGGSGGSGGSGGSCWARPCGDAANFYDWFVQQASKKAAAGAPVP YPDPLEPRPG; SEQ ID NO:2255
RP9-12-RP9	1-1	1-1	2.7E-06	N/A	ADYKDDDDKGSLDESFYDWFERQLGKK GGSGGSGGSGGS DDDKGSLDESFYDWFERQLGKK AAAGAPVPYPDPLEPRG; SEQ ID NO:2254
RP6-L-D8B12	2-6	1-2	5.0E-06	N/A	ADYKDTFYSCLASLLTGTPQPNRGPWERCRAAAGAPADYKDWLEQERAWIWCEKGSGCRAAAAGAPVPYPDPLE PRPG; SEQ ID NO:2263
RP31-IGF-12-RP30-IGF	6-2	2-1	4.4E-06	7.2E-08	ADYKDGVDEVRAWLWCQISGLGCESKKGGSGGSGGSGGSSFYSCLESLVNGGAERSDGQWEGCRAAAGAPVP YPDPLEPRPG; SEQ ID NO:2265
RP30-IGF-12-RP31-IGF	2-6	1-2	6.8E-05	1.1E-06	ADYKDSFYSCLESLVNGGAERSDGQWEGCRGGSGGSGGSGGSGVDEVRAWLWCQISGLGCESKKAAAGAPVP YPDPLEPRPG; SEQ ID NO:2264
D8B12-12-RP9	6-1	2-1	1.0E-06	N/A	ADYKDWLEQERAWIWCEKGSGCRAGGSGGSGGSGGSDDDKGSLDESFYDWFERQLGKKAAAGAPVPYPDPLE PRG; SEQ ID NO:2241

Monomer and dimer peptides which stimulate cell proliferation using the MiaPaCa cells. Form. = formula; N/A = not available; Misc. = miscellaneous sequence; Cysteine pairs are shaded and underlined; FY, WF, and L residues from Formula 1 and Formula 2 motifs are shaded and shown in bold. .

TABLE 29

IGF-1R Agonists in FDC-P2 Cells

Peptide	Form.	Site IGF-1R	Cellular EC_{50} (M)	Binding IC_{50} (M)	Sequence
G33-lig	1	1	3.0E-06	7.1E-07	GIISQSCPESFYDWFAGQVSDPWWCW-Lig SEQ ID NO:
G33	1	1	2.0E-06	6.0E-07	GIISQSCPESFYDWFAGQVSDPWWCW SEQ ID NO:2249
S175	1	1	1.0E-05	7.1E-06	GRVDWLQRNANFYDWFVAELG SEQ ID NO:2248
D815	6	2	3.0E-06	1.1E-05	WLDQERAWLWCEISGRGCLS SEQ ID NO:2252
lig-D815	6	2	1.0E-05	2.2E-06	Lig-WLDQERAWLWCEISGRGCLS SEQ ID NO:
RP31-IGF	6	2	2.0E-05	8.4E-07	GVDEVRAWLWCQISGLGCES SEQ ID NO:2253
D815	6	2	1.0E-06	1.5E-06	WLDQERAWLWCEISGRGCLS SEQ ID NO:2252
RP6-RP9	2-1	1-1	6.0E-06	N/A	ADYKDTFYSCLASLLTGTPQPNRGPWERCRGSLDESFYDWFERQLAAAGAPVPYPDPLEPRPG SEQ ID NO:2259
G33-6-G33	1-1	1-1	3.0E-06	1.4E-07	ADYKDGIISQSCPESFYDWFAGQVSDPWWCWGGSGGSGIISQSCPESFYDWFAGQVSDPWWCWKKA AAGAPVPYPDPLEPRPG; SEQ ID NO:2256
D815-RP9	6-1	2-1	3.0E-06	1.1E-06	WLDQERAWLWCEISGRGCLSGSLDESFYDWFERQLG SEQ ID NO:2260

Monomer and dimer peptides which stimulate cell proliferation using the FDC-P2 cells. Form. = formula; N/A = not available; Lig = Diaminopropionic acid with a 2-aminohydroxyacetyl group (CO-CH2-O-NH2) on the side chain amino group; Cysteine pairs are shaded and underlined; FY, WF, and L residues from Formula 1 and Formula 2 motifs are shaded and shown in bold.

TABLE 30

Peptide	Site IGFR/Formula	FDC-P2 Agonist	FDC-P2 Antagonist	MCF-7 Agonist	MCF-7 Antagonist	MiaPaCa Agonist	MiaPaCa Antagonist
Monomers:							
D815	2 (cys.) 6	+					
RP30-IGF	1 (cys.) 2		+	No	No		+
RP31-IGF	2 (cys.) 6	+		+		No	No
G33	1 (cys.) 1	+		+		+	
RP9	1 1		+	No	No	No	No
RP6	1 (cys.) 2		+		+	No	No
C1	1 (cys.) 1		+		+	No	No
RP33-IGF	1 (cys.) 2		+		+		+
H2C-A-H6	1 1	ND	ND		+		+
RP43	1 (cys.) 2		+	No	No		+
RP48	Misc. (cys.)	ND	ND	+		+	
RP52	Misc. (cys.)	ND	ND	No	No		+
RP54	Misc. (cys.)	ND	ND		+		+
RP56	Misc.	ND	ND	No	No		+
RP60	Misc. (cys.)	ND	ND	+		+	
Dimers:							
RP9-12-RP9	1-1 1-1	ND	ND	+		+	
D8B12-12-RP9	2-1 (cys.) 2-1	ND	ND	+		+	
RP9-L-RP6	1-1 (cys.) 1-2		+	+		+	
RP6-D8B12	1-2 (cys.) 2-2	ND	ND	+		+	

RP30-IGF-C1	1-1 (cys.) 2-1	ND	ND	+		+	
C1-RP30-IGF	1-1 (cys.) 1-1	ND	ND	+		+	
RP30-IGF-12-RP30-IGF	1-1 (cys.) 2-2	ND	ND		+		+
RP30-IGF-12-RP31-IGF	1-2 (cys.) 2-2	ND	ND	+		+	
RP31-IGF-12-RP30-IGF	2-1 (cys.) 2-2	ND	ND	+		+	
RP6-D8B12	1-2 (cys.) 2-2	ND	ND	+		+	

ND =Not Done; + = Effect observed; No = No effect observed; Cys. = contains putative cysteine loop.

Incorporated herein by reference in its entirety is the Sequence Listing for the application, comprising SEQ ID NO:1 to SEQ ID NO:2227. The Sequence Listing is disclosed on three CD-ROMs, designated "CRF", "Copy 1", and "Copy 2". The Sequence Listing is a computer-readable ASCII file named "18784056PC.app.txt", created on September 23, 2002, in IBM-PC machine format, on a MS-Windows®98 operating system. The 18784056PC.app.txt file is 927,477 bytes in size.

As various changes can be made in the above compositions and methods without departing from the scope and spirit of the invention, it is intended that all subject matter contained in the above description, shown in the accompanying drawings, or defined in the appended claims be interpreted as illustrative, and not in a limiting sense.

The contents of all patents, patent applications, published articles, books, reference manuals, texts and abstracts cited herein are hereby incorporated by reference in their entirety to more fully describe the state of the art to which the present invention pertains.

WHAT IS CLAIMED IS:

1. A method of modulating insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to modulate the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises a Formula 1 sequence, $X_1X_2X_3X_4X_5$, such that X_1, X_2, and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine; and ii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

2. The method of claim 1, wherein the amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

3. The method of claim 2, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence selected from the group consisting of:

a. H2C-A-H6 (SEQ ID NO:2228); and

b. RP9 (SEQ ID NO:2229).

4. The method of claim 2, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

a. S175 (SEQ ID NO:2248); and

b. 12-RP9ex (SEQ ID NO:2250).

5. The method of claim 2, wherein the amino acid sequence further comprises two or more cysteines which are separated by at least 3 amino acids.

6. The method of claim 5, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence selected from the group consisting of:

a. C1 (SEQ ID NO:2230);

b. C1KK (SEQ ID NO:2266); and

c. L-RP9ex (SEQ ID NO:2231).

7. The method of claim 5, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

a. G33 (SEQ ID NO:2249); and

b. L-RP9ex (SEQ ID NO:2231).

8. A method of decreasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to decrease the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises a Formula 2 sequence, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_6 and X_7 are aromatic amino acids, X_8, X_9, X_{11}, and X_{12} are any amino acid, and X_{10} and X_{13} are leucine; ii) the amino acid sequence further comprises two or more cysteines which are separated by at least 3 amino acids; and iii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

9. The method of claim 8, wherein the amino acid sequence decreases insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

10. The method of claim 9, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence selected from the group consisting of:

a. RP33-IGF (SEQ ID NO:2232);

b. RP6KK (SEQ ID NO:2233);

c. RP30-IGF (SEQ ID NO:2234);

d. RP43 (SEQ ID NO:2235);

e. RP33K-IGF (SEQ ID NO:2266); and

f. RP6 (SEQ ID NO:2236).

11. A method of increasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to increase the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises a Formula 6 sequence, X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}, such that X_{62}, X_{65}, X_{66} X_{68}, X_{69}, X_{71}, X_{73}, X_{76}, X_{77}, X_{78}, X_{80} and X_{81} are any amino acid, X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine, X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine, and methionine, X_{64} is selected from group consisting of aspartic acid and glutamic acid, X_{67} is tryptophan, X_{75} is selected from group consisting of tyrosine and tryptophan, and X_{72} and X_{79} are cysteines; and ii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

12. The method of claim 11, wherein the amino acid sequence increases insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

13. The method of claim 12, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

a. D815 (SEQ ID NO:2252); and

b. RP31-IGF (SEQ ID NO:2253).

14. A method of modulating insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to modulate the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises at least two Formula 1 subsequences, $X_1X_2X_3X_4X_5$, such that X_1, X_2, and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine; and ii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

15. The method of claim 14, wherein the amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

16. The method of claim 15, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence selected from the group consisting of:

a. RP9-RP9 (C-C; SEQ ID NO:2237);

b. RP9-RP9 (C-N; SEQ ID NO:2238); and

c. RP9-L-RP9 (SEQ ID NO:2239).

17. The method of claim 15, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence, RP9-12-RP9 (SEQ ID NO:2254).

18. The method of claim 15, wherein the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids.

19. The method of claim 18, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence, G33-RP9 (SEQ ID NO:2240).

20. The method of claim 18, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

a. D112-12-D112 (SEQ ID NO:2255); and

b. G33-6-G33 (SEQ ID NO:2256).

21. A method of decreasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to decrease the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises at least two Formula 2 subsequences, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_6 and X_7 are aromatic amino acids, X_8, X_9, X_{11}, and X_{12} are any amino acid, and X_{10} and X_{13} are leucine; ii) the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids; and iii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

22. The method of claim 21, wherein the amino acid sequence decreases insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

23. The method of claim 22, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence, RP30-IGF-12-RP30-IGF (SEQ ID NO:2241).

24. A method of modulating insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to modulate the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises a Formula 1 subsequence $X_1X_2X_3X_4X_5$, and a Formula 2 subsequence, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_1, X_2, and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, and such that X_6 and X_7 are aromatic amino acids, X_8, X_9, X_{11}, and X_{12} are any amino acid, and X_{10} and X_{13} are leucine; ii) the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids; iii) the subsequences are oriented Formula 1 to Formula 2; and iv) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

25. The method of claim 24, wherein the amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

26. The method of claim 25, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence, RP9-L-RP6 (SEQ ID NO:2242).

27. The method of claim 25, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

a. RP9-L-RP6 (SEQ ID NO:2242); and

b. D112-12-RP30-IGF (SEQ ID NO:2257).

28. A method of increasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to increase the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises a Formula 1 subsequence $X_1X_2X_3X_4X_5$, and a Formula 2 subsequence, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_1, X_2, and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, and such that X_6 and X_7 are aromatic amino acids, X_8, X_9, X_{11}, and X_{12} are any amino acid, and X_{10} and X_{13} are leucine; ii) the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids; iii) the subsequences are oriented Formula 2 to Formula 1; and iv) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

29. The method of claim 28, wherein the amino acid sequence increases insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

30. The method of claim 29, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

a. RP30-IGF-12-D112 (SEQ ID NO:2258); and

b. RP6-RP9 (SEQ ID NO:2259).

31. A method of decreasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to decrease the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises a Formula 1 subsequence $X_1X_2X_3X_4X_5$, and a Formula 6 subsequence, X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}, such that X_1, X_2, and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, and such that X_{62}, X_{65}, X_{66} X_{68}, X_{69}, X_{71}, X_{73}, X_{76}, X_{77}, X_{78}, X_{80} and X_{81} are any amino acid, X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; X_{75} is selected from group consisting of tyrosine and tryptophan, and X_{72} and X_{79} are cysteines; ii) the subsequences are oriented Formula 1 to Formula 6; and iii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

32. The method of claim 31, wherein the amino acid sequence decreases insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

33. The method of claim 32, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence, G33-D8B12 (SEQ ID NO:2243).

34. A method of modulating insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to modulate the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises a Formula 1 subsequence $X_1X_2X_3X_4X_5$, and a Formula 6 subsequence, X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}, such that X_1, X_2, and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, and such that X_{62}, X_{65}, X_{66} X_{68}, X_{69}, X_{71}, X_{73}, X_{76}, X_{77}, X_{78}, X_{80} and X_{81} are any amino acid, X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; X_{75} is selected from group consisting of tyrosine and tryptophan, and X_{72} and X_{79} are cysteines; ii) the subsequences are oriented Formula 6 to Formula 1; and iii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

35. The method of claim 34, wherein the amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

36. The method of claim 35, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence, D8B12-12-RP9 (SEQ ID NO:2244).

37. The method of claim 35, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

a. D8B12-12-RP9 (SEQ ID NO:2244); and

b. D815-RP9 (SEQ ID NO:2260).

38. A method of decreasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to decrease the activity of insulin-like growth factor receptor, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence selected from the group consisting of:

a. H2C-A-H6 (SEQ ID NO:2228);

b. RP9 (SEQ ID NO:2229);

c. C1 (SEQ ID NO:2230);

d. L-RP9ex (SEQ ID NO:2231);

e. RP33-IGF (SEQ ID NO:2232);

f. RP6KK (SEQ ID NO:2233);

g. RP30-IGF (SEQ ID NO:2234);

h. RP43 (SEQ ID NO:2235);

i. RP6 (SEQ ID NO:2236);

j. RP52 (SEQ ID NO:2245);

k. RP54 (SEQ ID NO:2246);

l. RP33K-IGF (SEQ ID NO:2266);

m. C1KK (SEQ ID NO:2266); and

n. RP56 (SEQ ID NO:2247).

39. A method of decreasing insulin-like growth factor receptor activity in activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to decrease the activity of insulin-like growth factor receptor, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence selected from the group consisting of:

a. RP9-RP9 (C-C; SEQ ID NO:2237);

b. RP9-RP9 (C-N; SEQ ID NO:2238);

c. RP9-L-RP9 (SEQ ID NO:2239);

d. G33-RP9 (SEQ ID NO:2240);

e. RP30-IGF-12-RP30-IGF (SEQ ID NO:2241);

f. RP9-L-RP6 (SEQ ID NO:2242);

g. G33-D8B12 (SEQ ID NO:2243); and

h. D8B12-12-RP9 (SEQ ID NO:2244).

40. A method of increasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to increase the activity of insulin-like growth factor receptor, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

a. S175 (SEQ ID NO:2248);

b. G33 (SEQ ID NO:2249);

c. 12-RP9ex (SEQ ID NO:2250);

d. L-RP9ex (SEQ ID NO:2231);

e. D815 (SEQ ID NO:2252);

f. RP31-IGF (SEQ ID NO:2253);

g. RP48 (SEQ ID NO:2261); and

h. RP60 (SEQ ID NO:2262).

41. A method of increasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to increase the activity of insulin-like growth factor receptor, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

a. RP9-12-RP9 (SEQ ID NO:2254);

b. D112-12-D112 (SEQ ID NO:2255);

c. G33-6-G33 (SEQ ID NO:2256);

d. RP9-L-RP6 (SEQ ID NO:2242);

e. D112-12-RP30-IGF (SEQ ID NO:2257);

f. RP9-L-RP6 (SEQ ID NO:2242);

g. RP30-IGF-12-D112 (SEQ ID NO:2258);

h. RP6-RP9 (SEQ ID NO:2259);

i. D8B12-12-RP9 (SEQ ID NO:2244);

j. RP6-L-D8B12 (SEQ ID NO:2263);

k. RP30-IGF-12-RP31-IGF (SEQ ID NO:2264);

l. RP31-IGF-12-RP30-IGF (SEQ ID NO:2265); and

m. D815-RP9 (SEQ ID NO:2260).

42. An insulin-like growth factor receptor modulator comprising an amino acid sequence which comprises a Formula 1 sequence, $X_1X_2X_3X_4X_5$, such that X_1, X_2, and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, wherein the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids; and with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

43. The insulin-like growth factor receptor modulator of claim 42, wherein amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

44. The insulin-like growth factor receptor modulator of claim 43, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence selected from the group consisting of:

a. C1 (SEQ ID NO:2230);

b. C1KK (SEQ ID NO:2266); and

c. L-RP9ex (SEQ ID NO:2231).

45. The insulin-like growth factor receptor modulator of claim 43, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

a. G33 (SEQ ID NO:2249); and

b. L-RP9ex (SEQ ID NO:2231).

46. An insulin-like growth factor receptor antagonist comprising an amino acid sequence which comprises a Formula 2 sequence, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_6 and X_7 are aromatic amino acids, X_8, X_9, X_{11}, and X_{12} are any amino acid, and X_{10} and X_{13} are leucine, wherein the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

47. The insulin-like growth factor receptor antagonist of claim 46, wherein amino acid sequence decreases insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

48. The insulin-like growth factor receptor antagonist of claim 47, wherein the amino acid sequence comprises a sequence selected from the group consisting of:

a. RP33-IGF (SEQ ID NO:2232);

b. RP6KK (SEQ ID NO:2233);

c. RP30-IGF (SEQ ID NO:2234);

d. RP43 (SEQ ID NO:2235);

e. RP33K-IGF (SEQ ID NO:2266); and

f. RP6 (SEQ ID NO:2236).

49. An insulin-like growth factor receptor modulator comprising an amino acid sequence which comprises at least two Formula 1 subsequences, $X_1X_2X_3X_4X_5$, such that X_1, X_2, and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, wherein the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

50. The insulin-like growth factor receptor modulator of claim 49, wherein amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

51. The insulin-like growth factor receptor modulator of claim 50, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence, G33-RP9 (SEQ ID NO:2240).

52. The insulin-like growth factor receptor modulator of claim 50, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

a. D112-12-D112 (SEQ ID NO:2255); and

b. G33-6-G33 (SEQ ID NO:2256).

53. An insulin-like growth factor receptor antagonist comprising an amino acid sequence that comprises at least two Formula 2 subsequences, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_6 and X_7 are aromatic amino acids, X_8, X_9, X_{11}, and X_{12} are any amino acid, and X_{10} and X_{13} are leucine, wherein the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

54. The insulin-like growth factor receptor antagonist of claim 53, wherein amino acid sequence decreases insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

55. The insulin-like growth factor receptor antagonist of claim 54, wherein amino acid sequence comprises the sequence RP30-IGF-12-RP30-IGF (SEQ ID NO:2241).

56. An insulin-like growth factor receptor modulator comprising an amino acid sequence which comprises a Formula 1 subsequence $X_1X_2X_3X_4X_5$, and a Formula 2 subsequence, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_1, X_2, and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, and such that X_6 and X_7 are aromatic amino acids, X_8, X_9, X_{11}, and X_{12} are any amino acid, and X_{10} and X_{13} are leucine, wherein the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids, and wherein the subsequences are oriented Formula 1 to Formula 2, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

57. The insulin-like growth factor receptor modulator of claim 56, wherein amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

58. The insulin-like growth factor receptor modulator of claim 57, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence, RP9-L-RP6 (SEQ ID NO:2242).

59. The insulin-like growth factor receptor modulator of claim 57, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

a. RP9-L-RP6 (SEQ ID NO:2242); and

b. D112-12-RP30-IGF (SEQ ID NO:2257).

60. An insulin-like growth factor receptor agonist comprising an amino acid sequence which comprises a Formula 1 subsequence $X_1X_2X_3X_4X_5$, and a Formula 2 subsequence, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_1, X_2, and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, and such that X_6 and X_7 are aromatic amino acids, X_8, X_9, X_{11}, and X_{12} are any amino acid, and X_{10} and X_{13} are leucine, wherein the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids, and wherein the subsequences are oriented Formula 2 to Formula 1, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

61. The insulin-like growth factor receptor agonist of claim 60, wherein amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

62. The insulin-like growth factor receptor agonist of claim 61, wherein the amino acid sequence comprises a sequence selected from the group consisting of:

a. RP30-IGF-12-D112 (SEQ ID NO:2258); and

b. RP6-RP9 (SEQ ID NO:2259).

63. An insulin-like growth factor receptor antagonist comprising an amino acid sequence selected from the group consisting of:

a. H2C-A-H6 (SEQ ID NO:2228);

b. L-RP9ex (SEQ ID NO:2231);

c. RP33-IGF (SEQ ID NO:2232);

d. RP30-IGF (SEQ ID NO:2234);

e. RP43 (SEQ ID NO:2235);

f. G33-RP9 (SEQ ID NO:2240);

g. RP30-IGF-12-RP30-IGF (SEQ ID NO:2241);

h. RP9-L-RP6 (SEQ ID NO:2242);

i. G33-D8B12 (SEQ ID NO:2243);

j. D8B12-12-RP9 (SEQ ID NO:2244);

k. RP52 (SEQ ID NO:2245);

l. RP54 (SEQ ID NO:2246);

m. RP33K-IGF (SEQ ID NO:2266); and

n. RP56 (SEQ ID NO:2247).

64. An insulin-like growth factor receptor agonist comprising an amino acid sequence selected from the group consisting of:

a. 12-RP9ex (SEQ ID NO:2250);

b. L-RP9ex (SEQ ID NO:2231);

c. RP31-IGF (SEQ ID NO:2253);

d. D112-12-D112 (SEQ ID NO:2255);

e. G33-6-G33 (SEQ ID NO:2256);

f. RP9-L-RP6 (SEQ ID NO:2242);

g. D112-12-RP30-IGF (SEQ ID NO:2257);

h. RP30-IGF-12-D112 (SEQ ID NO:2258);

i. RP6-RP9 (SEQ ID NO:2259);

j. D8B12-12-RP9 (SEQ ID NO:2244);

k. D815-RP9 (SEQ ID NO:2260);

l. RP48 (SEQ ID NO:2261); and

m. RP60 (SEQ ID NO:2262).

65. A method of identifying an insulin-like growth factor receptor modulator comprising:

a. contacting insulin-like growth factor receptor with an amino acid sequence to form a complex, wherein i) the amino acid sequence comprises a Formula 1 sequence, $X_1X_2X_3X_4X_5$, such that X_1, X_2, and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine; ii) the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids; and iii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof;

b. contacting the complex of (a) with a compound library;

c. identifying a compound which disrupts the complex of (a); and

d. determining whether the compound exhibits agonist or antagonist activity at insulin-like growth factor receptor, wherein this activity indicates identification of an insulin-like growth factor receptor modulator.

66. The method of claim 65, wherein the amino acid sequence comprises a sequence selected from the group consisting of:

a. C1 (SEQ ID NO:2230);

b. L-RP9ex (SEQ ID NO:2231);

c. G33 (SEQ ID NO:2249);

d. C1KK (SEQ ID NO:2266); and

e. L-RP9ex (SEQ ID NO:2231).

67. A method of identifying an insulin-like growth factor receptor modulator comprising:

a. contacting insulin-like growth factor receptor with an amino acid sequence to form a complex, wherein i) the amino acid sequence comprises a Formula 2 sequence, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_6 and X_7 are aromatic amino acids, X_8, X_9, X_{11}, and X_{12} are any amino acid, and X_{10} and X_{13} are leucine; ii) the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids; and iii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof;

b. contacting the complex of (a) with a compound library;

c. identifying a compound which disrupts the complex of (a); and

d. determining whether the compound exhibits agonist or antagonist activity at insulin-like growth factor receptor, wherein this activity indicates identification of an insulin-like growth factor receptor modulator.

68. The method of claim 67, wherein the amino acid sequence comprises a sequence selected from the group consisting of:

a. RP33-IGF (SEQ ID NO:2232);

b. RP6KK (SEQ ID NO:2233);

c. RP30-IGF (SEQ ID NO:2234);

d. RP43 (SEQ ID NO:2235);

e. RP33K-IGF (SEQ ID NO:2266); and

f. RP6 (SEQ ID NO:2236).

69. A method of identifying an insulin-like growth factor receptor modulator comprising:

a. contacting insulin-like growth factor receptor with an amino acid sequence to form a complex, wherein i) the amino acid sequence comprises a Formula 6 sequence, X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}, such that X_{62}, X_{65}, X_{66} X_{68}, X_{69}, X_{71}, X_{73}, X_{76}, X_{77}, X_{78}, X_{80} and X_{81} are any amino acid, X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; X_{75} is selected from group consisting of tyrosine and tryptophan, and X_{72} and X_{79} are cysteines; and ii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof;

b. contacting the complex of (a) with a compound library;

c. identifying a compound which disrupts the complex of (a); and

d. determining whether the compound exhibits agonist or antagonist activity at insulin-like growth factor receptor, wherein this activity indicates identification of an insulin-like growth factor receptor modulator.

70. The method of claim 69, wherein the amino acid sequence comprises a sequence selected from the group consisting of:

a. D815 (SEQ ID NO:2252); and

b. RP31-IGF (SEQ ID NO:2253).

71. A method of identifying an insulin-like growth factor receptor modulator comprising:

a. contacting insulin-like growth factor receptor with an amino acid sequence to form a complex,

b. contacting the complex of (a) with a compound library;

c. identifying a compound which disrupts the complex of (a); and

d. determining whether the compound exhibits agonist or antagonist activity at insulin-like growth factor receptor, wherein this activity indicates identification of an insulin-like growth factor receptor modulator, and wherein the amino acid sequence comprises a sequence selected from the group consisting of:

a. H2C-A-H6 (SEQ ID NO:2228);

b. RP9 (SEQ ID NO:2229);

c. C1 (SEQ ID NO:2230);

d. L-RP9ex (SEQ ID NO:2231);

e. RP33-IGF (SEQ ID NO:2232);

f. RP6KK (SEQ ID NO:2233);

g. RP30-IGF (SEQ ID NO:2234);

h. RP43 (SEQ ID NO:2235);

i. RP6 (SEQ ID NO:2236);

j. RP9-RP9 (C-C; SEQ ID NO:2237);

k. RP9-RP9 (C-N; SEQ ID NO:2238);

l. RP9-L-RP9 (SEQ ID NO:2239);

m. G33-RP9 (SEQ ID NO:2240);

n. RP30-IGF-12-RP30-IGF (SEQ ID NO:2241);

o. RP9-L-RP6 (SEQ ID NO:2242);

p. G33-D8B12 (SEQ ID NO:2243);

q. D8B12-12-RP9 (SEQ ID NO:2244);

r. RP52 (SEQ ID NO:2245);

s. RP54 (SEQ ID NO:2246);

t. RP33K-IGF (SEQ ID NO:2266);

u. C1KK (SEQ ID NO:2266); and

v. RP56 (SEQ ID NO:2247).

72. A method of identifying an insulin-like growth factor receptor modulator comprising:

a. contacting insulin-like growth factor receptor with an amino acid sequence to form a complex,

b. contacting the complex of (a) with a compound library;

c. identifying a compound which disrupts the complex of (a); and

d. determining whether the compound exhibits agonist or antagonist activity at insulin-like growth factor receptor, wherein this activity indicates identification of an insulin-like growth factor receptor modulator, and wherein the amino acid sequence comprises a sequence selected from the group consisting of:

a. S175 (SEQ ID NO:2248);

b. G33 (SEQ ID NO:2249);

c. 12-RP9ex (SEQ ID NO:2250);

d. L-RP9ex (SEQ ID NO:2231);

e. D815 (SEQ ID NO:2252);

f. RP31-IGF (SEQ ID NO:2253);

g. RP9-12-RP9 (SEQ ID NO:2254);

h. D112-12-D112 (SEQ ID NO:2255);

i. G33-6-G33 (SEQ ID NO:2256);

j. RP9-L-RP6 (SEQ ID NO:2242);

k. D112-12-RP30-IGF (SEQ ID NO:2257);

l. RP9-L-RP6 (SEQ ID NO:2242);

m. RP30-IGF-12-D112 (SEQ ID NO:2258);

n. RP6-RP9 (SEQ ID NO:2259);

o. D8B12-12-RP9 (SEQ ID NO:2244);

p. D815-RP9 (SEQ ID NO:2260);

r. RP48 (SEQ ID NO:2261);

s. RP6-L-D8B12 (SEQ ID NO:2263);

t. RP30-IGF-12-RP31-IGF (SEQ ID NO:2264);

u. RP31-IGF-12-RP30-IGF (SEQ ID NO:2265); and

v. RP60 (SEQ ID NO:2262).

73. A method of identifying an insulin-like growth factor receptor modulator comprising:

a. screening a library of amino acid sequences to isolate an amino acid sequence that binds to an insulin-like growth factor receptor, wherein the library is derived from a peptide sequence comprising at least one formula sequence selected from the group consisting of Formula 1, Formula 2, and Formula 6; and

b. determining whether the amino acid sequence isolated in (a) exhibits agonist or antagonist activity at insulin-like growth factor receptor in an insulin-like growth factor-responsive cell selected from the group consisting of FDC-P2, MCF-7, and MiaPaCa, wherein this activity indicates identification of an insulin-like growth factor receptor modulator.

74. A method of enhancing survival of an insulin-like growth factor-responsive mammalian cell comprising: contacting the cell with an amino acid sequence in an amount sufficient to enhance the survival of the cell, wherein the amino acid sequence is an insulin-like growth factor receptor agonist comprising at least one formula sequence selected from the group consisting of Formula 1, Formula 2, and Formula 6.

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
R40-3-40B2-IR	IRDMHYVWVQDRDRYINGVRQWYISDRYNPGSAFYRWFID	40.3	9.0	2.0	4.5	0.2
R40-4-40B12-IR	RMGLQALAHYRKSAGPIFLSSGSVIKGSEGDPFYAWFRLQ	60.4	12.9	2.0	6.5	0.2
R40-4-40G11-IR	MPVSLFRRVWDYRDGEHETLESHYVVPQAALDRLFYSWFS	52.6	37.5	2.0	18.8	0.1

FIG. 1A

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
R40-3-D5-IGFR	PLYGGGIHLYYPGTMGYVPGFPRQVKVLGDADKNFYDWFM	--	--	--	--	--
R40-3-A6-IGFR	YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV	--	--	--	--	--
R40-X-R35-IGFR	SGCCRLLGLRWMFIVIVGWSGALVCQSAASAAGFYDWFV	--	--	--	--	--

FIG. 1B

Clone Design	Sequence XXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
R20α-3-20D3-IR	IGGQGQHQDGNFYDWFVEALA	46.3	36.2	7.0	5.2	0.2
R20α-3-20F1-IR	VFWNCRSQQLDFYEWFEQAA	49.0	26.0	2.8	9.3	0.1
R20α-3-20H1-IR	RVAGAISAPGLVSNKQDGLFYSWFRE	45.6	35.3	3.3	10.7	0.1
R20α-3-20D1-IR	VLQARHGCDSVSDCFYEWFA	50.8	37.5	3.0	12.5	0.1
R20β-4-B12-IR	GAFYRWFHEALVGSERVPDV	41.9	2.9	5.7	0.5	2.0
R20β-4-H3-IR	HEAFYDWFSALVDGGYELMG	13.9	5.8	2.4	2.4	0.4
R20β-4-D10-2-IR	RIGGGWARSEGFYEWFVREL	21.5	7.3	2.9	2.5	0.4
R20β-4-C8-IR	LPAGGA?GFA?RGFYEWFES	44.9	31.1	9.6	3.2	0.3
R20β-4-E7-IR	GHSWALVRHVDRLFYEWFDL	45.0	18.8	5.9	3.2	0.3
R20β-4-E7-2-IR	LGTSAGQGVGHRAFYQWFQS	45.0	18.8	5.9	3.2	0.3
R20β-4-G3-IR	RGGGTFYEWFESALRKHGAG	38.6	7.5	2.0	3.8	0.3
R20β-4-H6-IR	NSSGQQVVGLTFYSWFASQV	14.8	7.6	2.0	3.8	0.3
R20β-4-G11-IR	FYGWFSRQLSLTPRDDWGLP	39.4	7.5	1.9	3.9	0.3
R20β-4-G8-IR	RMFYEWFWSQMGAGPTEGSA	41.2	15.1	3.4	4.4	0.2
R20β-4-H9-IR	IGGQGQHQDGNFYDWFVEALA	43.1	8.8	2.0	4.4	0.2
R20β-4-H8-IR	RDKPTDQEEQNWSFYEWFRH	47.9	43.7	9.3	4.7	0.2
R20β-4-B8-IR	WSALLSVMDTGFYAWFDDAV	44.0	40.1	8.4	4.8	0.2
R20β-4-E2-IR	SRDQTNFTFNSAGFYGWFER	16.3	13.9	2.4	5.8	0.2
R20β-4-F4-IR	GVGTLTMSSDAFYTWFV	15.3	5.9	1.0	5.9	0.2
R20β-4-A8-IR	IGGSFVEFYGWFNDQV	43.3	36.0	6.0	6.0	0.2
R20β-4-C4-IR	DIGSDGHGRRWDSFYRWFEM	17.3	26.8	4.3	6.2	0.2
R20β-4-D7-IR	VLQARHGCDSVSDCFYEWFA	44.8	36.2	5.6	6.5	0.2
R20β-4-D2-IR	DPERMQSDVGFYEWFRAAVG	31.2	29.4	2.9	10.1	0.1

FIG. 1C

Clone	Sequence	Ratios over Background			Comparisons	
Design	XXXXXXXXXXXXXXXXXXXX	E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
		--	--	--	--	--
R20-4-B9-IGFR	DPERMQSDVGFYEWFRAAVG	40.1	16.6	--	--	--
R20-4-F8-IGFR	DIGSDGHGRRWDSFYRWFEM	39.2	13.9	--	--	--
R20-4-G12-IGFR	PFYQWFLDQSVGGSRGGGLR	36.7	8.0	--	--	--
R20-4-D10-IGFR	AVAPLSVRGRDSGFYSWFSS	40.2	4.1	--	--	--

FIG. 1D

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXNFYDWFVXXXX	--	--	--	--	--
A6S-3-E12-IR	GRVDWLQRNANFYDWFVAELG	26.2	1.3	8.0	0.2	6.2
A6S-2-C1-IR	RMYFSTGAPQNFYDWFVQEWD	41.2	1.3	7.0	0.2	5.4
A6S-1-A7-IR	HHTQGLQVQRNFYDWFVNELR	47.2	2.3	11.1	0.2	4.8
A6S-2-C8-IR	MHRMQHDGTSNFYDWFVLQWA	44.9	1.5	5.5	0.3	3.7
A6S-3-E10-IR	AMHVVAQGGPNFYDWFVRELR	46.9	1.6	5.0	0.3	3.1
A6S-2-D5-IR	AIQMNGNLAFNFYDWFVRELT	31.9	1.2	3.7	0.3	3.1
A6S-1-B2-IR	TDRKSVQEPRNFYDWFVWAAR	31.6	1.8	5.3	0.3	2.9
A6S-1-A4-IR	PHGHRGFAQSNFYDWFVTQEE	43.3	3.6	9.2	0.4	2.6
A6S-4-G3-IR	RLASASVPGQNFYDWFVDQLL	31.3	2.3	5.1	0.5	2.2
A6S-4-H8-IR	RQSEFSTLNSNFYDWFVRELE	11.5	1.7	3.6	0.5	2.1
A6S-3-E11-IR	GQAQLSIRDVNFYDWFVQQLV	26.3	2.3	4.4	0.5	1.9
A6S-1-A1-IR	MSEPAVGVNGNFYDWFVAQLF	36.9	3.7	6.5	0.6	1.8
A6S-2-C9-IR	VGTGRARLDRNFYDWFVGQYS	43.6	1.3	2.3	0.6	1.8
A6S-2-C4-IR	SREAVQKRNANFYDWFVQQLS	34.5	5.6	9.6	0.6	1.7
A6S-4-H10-IR	LAQFAGSRNQNFYDWFVEQLG	39.2	4.4	6.9	0.6	1.6
A6S-4-G7-IR	GQEYFDQMGLNFYDWFVRELD	19.1	1.4	2.2	0.6	1.6
A6S-4-H2-IR	RQPSQPPHGSNFYDWFVEAIN	25.5	2.6	3.9	0.7	1.5
A6S-2-C3-IR	LMQSLGSGSTNFYDWFVQQMV	31.1	1.6	2.4	0.7	1.5
A6S-2-C11-IR	DQQRSACDGTNFYDWFVCQLS	20.9	3.3	4.6	0.7	1.4
A6S-3-F3-IR	LDGTKACQRVNFYDWFVCQTE	37.1	3.0	4.2	0.7	1.4
A6S-3-E5-IR	PEARRTVVHSNFYDWFVAQLS	31.6	2.5	3.5	0.7	1.4
A6S-1-B7-IR	PWMLSVGIQDNFYDWFVGLDS	49.2	1.6	2.3	0.7	1.4
A6S-3-E7-IR	ASHQRGGSSDNFYDWFVAQMR	37.2	5.0	6.3	0.8	1.3
A6S-4-G6-IR	TLEREGEFSGNFYDWFVEQLH	16.8	3.1	4.0	0.8	1.3
A6S-2-C2-IR	DRQSIGSVHGDFYDWFVSALG	29.7	2.4	3.1	0.8	1.3
A6S-3-F1-IR	DWDKLGSLSENFYDWFVDQLA	29.7	2.3	3.0	0.8	1.3
A6S-2-C5-IR	VRVVLNQSGRNFYDWFVIQLE	42.9	6.1	7.0	0.9	1.1
A6S-3-E4-IR	MASWQSRTPDNFYDWFVRELS	20.9	2.1	2.3	0.9	1.1

FIG. 1E-1

Clone Design	Sequence XXXXXXXXXXNFYDWFVXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
A6S-3-E9-IR	TTCHPRGEDCNFYDWFVLQLR	36.6	9.0	8.9	1.0	1.0
A6S-3-E1-IR	VRGNDSVLRANFYDWFVDQLS	36.7	6.8	6.9	1.0	1.0
A6S-4-H12-IR	TPRSQVRSDHNFYDWFVYQLA	46.3	6.1	5.8	1.1	1.0
A6S-2-D3-IR	ESLTGSRPDRNFYDWFVQQTS	37.0	5.3	5.1	1.0	1.0
A6S-3-E8-IR	PQSLTEVRTGNFYDWFVVQLH	42.7	5.2	5.1	1.0	1.0
A6S-1-A12-IR	DVGMGRVKETNFYDWFVRQLI	39.7	2.1	2.1	1.0	1.0
A6S-4-H3-IR	GADDIRSLNTNFYDWFVNQLS	18.6	3.1	2.9	1.1	0.9
A6S-3-F7-IR	GVSIQAGYKTNFYDWFVEAVR	46.2	2.3	2.1	1.1	0.9
A6S-2-D8-IR	VGEHRQMSVGNFYDWFVMQIA	31.2	2.0	1.7	1.2	0.9
A6S-3-F10-IR	GSSLGRSGPGNFYDWFVDQLE	39.0	5.9	4.5	1.3	0.8
A6S-4-G11-IR	HRQQDVVRQGNFYDWFVQALE	44.8	4.3	3.3	1.3	0.8
A6S-2-D2-IR	QDTFLTAREGNFYDWFIRALE	33.5	3.6	2.7	1.3	0.8
A6S-4-G8-IR	EAIMREEGQANFYDWFVRQLE	11.1	2.5	1.9	1.3	0.8
A6S-4-H6-IR	VCDVSTGGGTNFYDWFVCQVG	22.4	2.4	1.9	1.3	0.8
A6S-2-D10-IR	PQPRSASTPLNFYDWFVQATG	41.3	2.1	1.7	1.2	0.8
A6S-3-F4-IR	GVSRGSGGDPNFYDWFVMQLR	37.0	13.5	9.9	1.4	0.7
A6S-4-G9-IR	GPGRHDSSRGNFYDWFVEQLA	36.2	11.8	7.8	1.5	0.7
A6S-3-F5-IR	ERFALEVQGSNFYDWFVRQVI	48.1	7.2	4.8	1.5	0.7
A6S-4-H1-IR	NLKSSATVGGNFYDWFVEQL	18.3	3.6	2.6	1.4	0.7
A6S-3-F6-IR	MEGPPAGGPLNFYDWFVAQVD	18.7	2.9	1.9	1.5	0.7
A6S-3-F11-IR	RLDVAGHRGGNFYDWFVKQLH	33.8	2.0	1.4	1.4	0.7
A6S-2-C6-IR	PWSDHEALNQNFYDWFVSQVL	46.7	19.2	12.1	1.6	0.6
A6S-4-G4-IR	EDRLGNGESTNFYDWFVRQLA	36.9	18.2	10.7	1.7	0.6
A6S-4-G12-IR	GKLVASTLDDNFYDWFVRQLS	32.8	12.8	7.9	1.6	0.6
A6S-2-D7-IR	SGPVVQTQNGNFYDWFVHQLR	33.2	12.0	7.1	1.7	0.6
A6S-4-G10-IR	VDRAGPAGSDNFYDWFVAQLD	33.9	10.8	6.8	1.6	0.6
A6S-3-F9-IR	SLGRNDRPDENFYDWFVSQVQ	44.3	9.6	5.7	1.7	0.6
A6S-3-F2-IR	RVMATANAPMNFYDWFVVQLQ	23.2	4.3	2.5	1.7	0.6

FIG. 1E-2

Clone Design	Sequence XXXXXXXXXXNFYDWFVXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
A6S-4-G1-IR	NGVERAGTGDNFYDWFVAQLH	36.2	31.8	15.7	2.0	0.5
A6S-1-A3-IR	PFAGKGDKTGNFYDWFVSLTG	39.9	12.6	6.0	2.1	0.5
A6S-3-F12-IR	GMPQEYMDQVNFYDWFVAQVD	41.4	7.4	4.0	1.9	0.5
A6S-4-G2-IR	MGTPAVGDGANFYDWFVRQLG	26.7	7.0	3.5	2.0	0.5
A6S-1-B1-IR	SKCKAWYGANNFYDWFVWQVD	30.6	3.7	1.9	1.9	0.5
A6S-2-D11-IR	EAASLGSQDRNFYDWFVRQVV	48.4	37.4	13.5	2.8	0.4
A6S-2-D1-IR	VERSASSQDGNFYDWFVVQIR	37.8	30.6	12.0	2.6	0.4
A6S-3-E2-IR	TSEVQRRSQDNFYDWFVAQVA	33.1	24.7	9.8	2.5	0.4

FIG. 1E-3

Clone Design	Sequence XXXXXXXXXXXNFYDWFVXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
A6S-4-E4-IGFR	ERSAAGFREGNFYDWFVAQVN	27	32	--	--	--
A6S-2-D2-IGFR	RAERGSMRDSNFYDWFVQQLP	36	30	--	--	--
A6S-2-F2-IGFR	LAMSVASRPANFYDWFVAQIV	35	30	--	--	--
A6S-4-F3-IGFR	HNSSSPMRTGNFYDWFVQELR	26	30	--	--	--
A6S-4-G4-IGFR	SALSGPVQPINFYDWFVTGM	26	30	--	--	--
A6S-4-G3-IGFR	GAQAIREIHHNFYDWFVAQVT	21	29	--	--	--
A6S-2-H2-IGFR	RGQRESDSGTNFYDWFVGAIR	40	28	--	--	--
A6S-2-E3-IGFR	VQEGLSGMEGNFYDWFVDQLF	36	28	--	--	--
A6S-4-C6-IGFR	RLDRSSTSGVNFYDWFVAQVG	25	28	--	--	--
A6S-4-F5-IGFR	GSQHSGREPHNFYDWFVAQVG	24	28	--	--	--
A6S-4-H3-IGFR	GRGDQRHETTNFYDWFVRELQ	20	28	--	--	--
A6S-4-H4-IGFR	PRMVEKPSEDNFYDWFVTQLS	20	28	--	--	--
A6S-2-H1-IGFR	RVGIQVDPHTNFYDWFVIQLT	42	27	--	--	--
A6S-4-E6-IGFR	RSSGGLLSQGNFYDWFVSQLE	24	26	--	--	--
A6S-4-B6-IGFR	SDARQAGLQENFYDWFVSQVR	23	26	--	--	--
A6S-4-D2-IGFR	PPYRSSRLGENFYDWFVMQVR	19	26	--	--	--
A6S-4-G5-IGFR	QEVTRTRDDKNFYDWFVSQIF	18	26	--	--	--
A6S-2-A3-IGFR	SRAPYGSTAGNFYDWFVQAVS	37	25	--	--	--
A6S-4-E2-IGFR	?DGQSVSSKGNFYDWFVQQMT	25	25	--	--	--
A6S-4-G6-IGFR	RLMGGIAEPQNFYDWFVREVA	20	25	--	--	--
A6S-4-G2-IGFR	SAGHHMPRESNFYDWFVDQVV	25	24	--	--	--
A6S-4-D6-IGFR	LGAAETWDGINFYDWFVKQVS	22	24	--	--	--
A6S-4-F4-IGFR	VGHSGVPPYPNFYDWFVMQVS	22	24	--	--	--
A6S-4-C3-IGFR	VTMLDKGAQDNFYDWFVREVA	21	24	--	--	--
A6S-4-H5-IGFR	HHSPGNEHGYNFYDWFVLQVA	19	24	--	--	--
A6S-4-H6-IGFR	GSIAQLIMRANFYDWFVEQTN	18	24	--	--	--
A6S-4-F6-IGFR	LKGSSQPLSVNFYDWFVQQIK	17	24	--	--	--
A6S-3-H1-IGFR	PASNKNSLAENFYDWFVQQTR	30	23	--	--	--

FIG. 1F-1

Ratios over Background		Comparisons				
Clone	Sequence	E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXNFYDWFVXXXX	--	--	--	--	--
A6S-4-A6-IGFR	HVEHMAVGDGNFYDWFVVQLR	21	23	--	--	--
A6S-4-E3-IGFR	RGMTGMVGRGNFYDWFVGQLR	21	23	--	--	--
A6S-4-D3-IGFR	GLRSEQGNRLNFYDWFVAQIA	20	23	--	--	--
A6S-3-E10-IGFR	RVREKLPRPENFYDWFVNQIH	23	22	--	--	--
A6S-4-D1-IGFR	SNPSRQDASVNFYDWFVREVA	22	22	--	--	--
A6S-4-B2-IGFR	QSVDLSRPDSNFYDWFVEVLS	21	22	--	--	--
A6S-4-A2-IGFR	IGGQGQHQDGNFYDWFVEALA	20	22	--	--	--
A6S-4-A5-IGFR	VEVQRHIRKDNFYDWFVKQID	19	22	--	--	--
A6S-4-C1-IGFR	CWARPCGDAANFYDWFVQQAS	16	22	--	--	--
A6S-4-B1-IGFR	RHERGKEGPGNFYDWFVSQVV	19	21	--	--	--
A6S-4-B4-IGFR	ERSPRPALASNFYDWFVQQVV	19	21	--	--	--
A6S-4-D4-IGFR	IARMRETFQPNFYDWFVDQLA	18	21	--	--	--
A6S-3-F8-IGFR	GRGQGLKRPDNFYDWFVAAAK	25	20	--	--	--
A6S-3-H9-IGFR	YSIEVQDWNENFYDWFVSQLG	23	20	--	--	--
A6S-3-G2-IGFR	TWMWEERKQDNFYDWFVGQLK	21	20	--	--	--
A6S-4-H2-IGFR	VTFTSAVFHENFYDWFVRQVS	19	20	--	--	--
A6S-4-A3-IGFR	LAINDLVTHKNFYDWFVDQLR	18	20	--	--	--
A6S-3-G10-IGFR	GAVGLAEAGPNFYDWFVSQVQ	24	19	--	--	--
A6S-3-E5-IGFR	RYRGERHDGRNFYDWFVEQVN	21	19	--	--	--
A6S-3-H2-IGFR	QGAEGRLSEGNFYDWFVQAVS	21	19	--	--	--
A6S-3-G3-IGFR	PRLHMGSDMGDFYDWFVVQIA	21	18	--	--	--
A6S-4-H1-IGFR	IVAGARHSEVNFYDWFVIQVR	18	18	--	--	--
A6S-4-G1-IGFR	AELVGAGVRGNFYDWFVDQLV	16	16	--	--	--
A6S-4-A1-IGFR	DSSRLWLGERNFYDWFVAQIS	17	12	--	--	--
A6S-2-F1-IGFR	VGQVGRYVRSNFYDWFVQQAM	30	8	--	--	--
A6S-2-G1-IGFR	RPQLVESGSKNFYDWFVQVVR	30	8	--	--	--
A6S-1-C5-IGFR	RIHNQTERGGNFYDWFVHQLV	27	7	--	--	--
A6S-2-B2-IGFR	EMYGDTSERVNFYDWFVSALQ	30	5	--	--	--

FIG. 1F-2

Ratios over Background		Comparisons				
Clone	Sequence	E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXNFYDWFVXXXX	--	--	--	--	--
A6S-1-D5-IGFR	RVGSGMEDLGNFYDWFVRQAQ	25	5	--	--	--
A6S-1-A2-IGFR	KDPVTVSQGRNFYDWFVVQIQ	20	5	--	--	--
A6S-3-E6-IGFR	DARDHGVWVMSNFYDWFVAQVS	20	5	--	--	--
A6S-1-G3-IGFR	VATVHVGGGMNFYDWFVAQVG	19	5	--	--	--
A6S-3-G4-IGFR	CADPGACSSLNFYDWFVQMRG	21	4	--	--	--
A6S-3-H8-IGFR	NPTSVQQYGVNFYDWFVNVLS	20	4	--	--	--
A6S-3-E3-IGFR	RPSLPEVRPGNFYDWFVQSVR	19	4	--	--	--
A6S-3-D9-IGFR	SLQGADFQQGNFYDWFVSELA	17	4	--	--	--
A6S-2-A1-IGFR	LSSRGRVTMRNFYDWFVAQVV	31	3	--	--	--
A6S-1-H4-IGFR	HKSWTTMSPLNFYDWFVAQVE	18	3	--	--	--
A6S-3-C1-IGFR	RPVIGGGGTRNFYDWFVAQMI	17	3	--	--	--
A6S-3-B10-IGFR	YDQDPPYWGLNFYDWFVREVA	16	3	--	--	--

FIG. 1F-3

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	**YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV**	**19.0**	**.4.0**	--	--	--
A6L-3-D1-IR	QRGMLVRGRISHGAGKIAYEPPDCLGQKACAVNFYDWFV	22.6	19.8	26.5	0.7	1.3
A6L-4-H7-IR	QRGMLLLGRISDDAGKVASEPSARRGQKVFAFNFYDWFV	37.5	3.5	4.2	0.8	1.2
A6L-4-H4-IR	YRGILVLGRISEGAGKVASEPAARIGQKVFADFYDWFV	38.5	21.1	25.8	0.8	1.2
A6L-4-E4-IR	QRGMLALGRISDGAGKVASEPPAGIGQKVFAFNFYDWFV	38.1	5.4	6.0	0.9	1.1
A6L-4-G7-IR	FRGRLVLGHFSDGAGKVGSEPAARIGQKVFDVNFYDWFV	38.6	16.2	18.5	0.9	1.1
A6L-3-C3-IR	YRGMLVLGRISDGAGKVASEPPARIGQEVFADNFYDWFV	34.7	21.8	23.1	0.9	1.1
A6L-3-B6-IR	YRGMLVLGRISDGAGEVASEPPARIGQEVFALNFYDWFV	33.1	27.8	30.3	0.9	1.1
A6L-4-G11-IR	VPWYAGSGSSSDGAGKVASEPPARIDQKVFAVNFYDWFV	27.6	2.0	2.0	1.0	1.0
A6L-4-G12-IR	YRGQLVLGRISYGAGKVGCDPPARIGQKDWAVNFYDWFV	32.0	2.3	2.3	1.0	1.0
A6L-3-A10-IR	QRGLLVLGRFSDGAGNVASEPPAGIGQEVFPVNFYDWFV	21.1	2.4	2.4	1.0	1.0
A6L-4-E12-IR	QRGMLVLGRISDGAGKVAAEPPDCLGQKVCAVNFYDWFV	3.1	2.4	2.4	1.0	1.0
A6L-4-E10-IR	QRGMRVLGRISDGAGKVASELPPRIGQKDFAVNFYDWFV	30.1	3.8	3.8	1.0	1.0
A6L-4-G8-IR	QRGMLVLGSISDGAGKVAYEAPARIGQTVFAVNFYDWFV	37.9	4.7	4.7	1.0	1.0
A6L-3-C12-IR	QPWCAGSGRIYDGACKVASEPPAHIGQEVFAVNFYDWFV	29.5	5.7	5.7	1.0	1.0
A6L-4-H11-IR	QRGMLVLDRISDGAGKVASGPPARIGQNVLAVNFYDWFV	35.4	9.6	9.6	1.0	1.0
A6L-4-F10-IR	YRGMLVVGRISDGTGKVASQPPARIGQKVFAVNFYDWFV	31.6	10.5	10.5	1.0	1.0
A6L-4-E9-IR	YRGMLVLGRISDGAGKVASVPPAHIGQKVFAFNFYDWFV	39.8	12.9	12.9	1.0	1.0
A6L-4-H8-IR	QHGMLVLGRVSVGAGKVPSEPQARIGHKVFDVNFYDWFV	38.2	14.6	14.6	1.0	1.0
A6L-3-A11-IR	YSGYAGSGSFSDGAGKVASEPPARISQEVLADNFYDWFV	29.0	17.5	17.5	1.0	1.0
A6L-4-F9-IR	YRGMLVLGRISDGAGKVASEPPARIGQKVSAVNFYDWFV	35.7	18.4	18.4	1.0	1.0
A6L-4-G2-IR	YHGKLDLGRISVGVGKVASEPPARIGQKVFADNFYDWFV	29.5	21.4	20.7	1.0	1.0
A6L-4-E8-IR	YRGQAGSGVGSLTVAGKVASDPPARIGQKVFADNFYDWFV	28.7	21.6	21.6	1.0	1.0
A6L-4-H10-IR	HRGMLVLGRISEGAGNVDPEPPARIGQNVFAGNFYDWFV	30.0	22.1	22.1	1.0	1.0
A6L-4-G9-IR	QRGMPVLGRISDGAGKVGSEPPARIARKVFPVNFYDWFI	37.1	22.6	22.6	1.0	1.0
A6L-4-F7-IR	QGGLLVTGRISDGAGKVASEPPGGIGQKVFAGNFYDWFV	28.6	23.6	24.4	1.0	1.0
A6L-4-E11-IR	YPWYGGSGTYLDGAGKVASEPPARIDQQVFAGNFYDWFV	38.4	26.5	26.5	1.0	1.0

FIG. 1G-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	**YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV**	**19.0**	**.4.0**	**--**	**--**	**--**
A6L-4-H9-IR	YRAMLVLRRISDVAGIVDSEPPTRIGQKVFAGNFYDWFV	37.5	27.3	27.3	1.0	1.0
A6L-4-E1-IR	YRGMLVLGRISQGAGNVASEPSSRIGQKVFAGNFYDWFI	35.4	32.6	31.4	1.0	1.0
A6L-3-A5-IR	YRGMLVLGRISDGAGKVDYEPPARIGQKVFAGNFYDWFV	38.3	34.6	35.5	1.0	1.0
A6L-4-G4-IR	YRGMLGLGGISAGAGIVASEPPARVGQKVFAGNFYDWFV	30.4	17.7	15.2	1.2	0.9
A6L-4-H2-IR	YRGILFQGRIPDGAGKVASEPPTRIGERVFAVNFYDWFV	36.1	4.2	3.6	1.1	0.9
A6L-4-E6-IR	QGGMPVLGRISDGAGKVAFEPPARIGQKVFAGNFYDWFV	28.6	24.1	22.7	1.1	0.9
A6L-4-H5-IR	YRGMLVLGRIQDGAGKVASEPPARIGQKVFTGNFYDWFV	37.2	24.6	23.1	1.1	0.9
A6L-4-H3-IR	QRGMLVLGGVSDGAGKVASDPPASIGQNVFAVNFYDWFV	37.1	9.1	7.2	1.3	0.8
A6L-4-E5-IR	YPGMLILDRISDGASKVVSEPPASIGQKVFAVNFYDWFV	42.1	30.6	24.4	1.3	0.8
A6L-3-C5-IR	YRGMLVLDRISDGAGKVASEQPARIGQEVYAVNFYDWFV	42.2	21.9	17.5	1.2	0.8
A6L-4-G6-IR	YRGMLDLGRISGGVGKVASESPARIGQKVYAVNFYDWFV	29.8	4.3	2.8	1.5	0.7
A6L-3-D4-IR	QRGMMVLGRISDGAGEVASEKVFAVNFYDWFV	39.9	12.4	8.4	1.5	0.7
A6L-3-A7-IR	QRGMLVLGRVSDGAGKVDSAPPARIGQKVFAGNFYDWFV	31.0	21.2	14.0	1.5	0.7
A6L-3-A6-IR	QRGMLVLGRMSDGAGKVAFEPPARIGQRGFAGNFYDWFV	25.5	12.3	8.8	1.4	0.7
A6L-4-E7-IR	QRGTLVLGRISDGAGKAASEPPARIGQNVFAVNFYDWFV	38.4	12.5	7.1	1.7	0.6
A6L-3-C6-IR	QRGMLVLDRISDGAGKVAAEPPARIGQKVFALNFYDWFI	28.8	10.9	6.7	1.6	0.6
A6L-4-F5-IR	QRGMLVLGRISDGAGEVASEPPARIGEKVYAVNFYDWFV	33.8	6.3	4.1	1.5	0.6
A6L-3-B7-IR	QRGILVRGRISDGAGKVGSEPPARSGEKVFAVNFYDWFI	27.6	9.4	5.0	1.9	0.5
A6L-4-F4-IR	QLGMVVLGRISDGSGKAASEPAARISQKVFAVNFYDWFV	38.9	17.6	9.4	1.9	0.5
A6L-4-E3-IR	QRGMLVLGRISDGDGKVASEPPARIGQRVFAVNFYDWFV	38.0	6.9	3.8	1.8	0.5
A6L-0-E6-IR	YRGMLVLGRSSDGAGKVAFERPARIGQTVFAVNFYDWFV	31.0	31.0	1.8	17.0	0.1
A6L-0-E4-IR	YRGMLVLGRISDGAG#VASEPPARIGRKVFAVNFYDWFV	26.0	16.0	1.3	13.0	0.1
A6L-0-H3-IR	YRGMLVLGRISGGAGKAASERPARIGQKVSAVNFYDWFV	27.0	26.0	2.0	13.0	0.1

FIG. 1G-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	YRGMLVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV	19	4	--	--	--
A6L-4-F8-IGFR	YRGMMVQGRISDGAGKVASVSPVRIGQKVIAVNFYDWFV	26	28	--	--	--
A6L-2-G9-IGFR	YRGRLGLGRISDVAGKVACDPSARIGQKVLPVNFYDWFV	39	22	--	--	--
A6L-4-E7-IGFR	YRGMLVLGRISDGAGRVASEPQARIGQKVFAVNFYDWFV	23	22	--	--	--
A6L-4-G10-IGFR	QGGMLVPGRISDGAGKVASQPPARIGPKGFAGNFYDWFV	19	22	--	--	--
A6L-2-E9-IGFR	YRGMRVLGRISDGAGKVASEPPTHIGQKVFPVNFYDWFV	38	21	--	--	--
A6L-2-D6-IGFR	YRGMLVLGRISDGAGKVGSEPAARIGQKVFALNFYDWFV	34	21	--	--	--
A6L-3-H12-IGFR	YRGQGMVLGRISDGAGKVASEPPGRIGQKVFPVNFYDWFV	24	21	--	--	--
A6L-4-A7-IGFR	YRGMLGLGRITGGAGKVASEPPDRIGQHVFVDNFYDWFV	20	20	--	--	--
A6L-4-B8-IGFR	DGMLVLGRISDGAGNVASEAPARIGQKVFAVNFYDWFV	20	19	--	--	--
A6L-4-G7-IGFR	YRGMRVRGRISDGAGKAASDPRARIGQTVLDVNFYDWFV	19	19	--	--	--
A6L-2-D9-IGFR	YRGMWVLGRISYGAGKVAYEPPARMGQKGFAVNFYDWFV	38	18	--	--	--
A6L-4-F7-IGFR	YRGMLVGGRIAGGAGIVASEPPARIGQKVFAVNFYDWFV	18	18	--	--	--
A6L-4-E12-IGFR	YRGLLGLGGISDGAGKVASEPPARNGQKVFAVNFYDWFV	15	13	--	--	--
A6L-4-H7-IGFR	YRGMLGLGRISAGAGKVASGAPARIGQEDFAVNFYDWFV	14	13	--	--	--
A6L-4-H12-IGFR	YRGMLALGRISEGAGKVASEPPARIGQNVFAVNFYDWFV	13	12	--	--	--
A6L-2-A4-IGFR	YRGMLVLGRISDGAGKVASEPPARIGQKVLAVNFYDWFV	17	4	--	--	--
A6L-3-D10-IGFR	YPGMLVPGRISDGAGEGATDPPPRIGQKVFAFNFYDWFV	16	4	--	--	--
A6L-2-F6-IGFR	YRGMLVPGRISDGAGKVAYEPPARIGQKIFAVNFYDWFV	15	4	--	--	--
A6L-2-B11-IGFR	YRGVLVLGRVSDGVGKVASEPPAHRGQRVFGVNFYDWFV	26	3	--	--	--
A6L-1-B7-IGFR	YRRMLVLGRISDGAANVASGPPDRIGQKVFAGNFYDWFV	23	3	--	--	--
A6L-1-D8-IGFR	YRRMLALGRFSDVTGDVASEPPAHIGQKVVAVNFYDWFV	23	3	--	--	--
A6L-0-A11-IGFR	YRGMVVRGRIFDGPGKVASEPRARIGQKVFAVNFYDWFV	19	3	--	--	--
A6L-3-B7-IGFR	YRGMLILGRISDGAGKVASEPPARVGQDVVAVNFYDWFV	9	3	--	--	--
A6L-1-G7-IGFR	YPGRLVGGRISDGVGKVASEPPGRIGQKVFAVNFYDWFV	20	2	--	--	--
A6L-1-B9-IGFR	QRGLLVLGRIFDGAGKVASDPPARIGQKDFADNFYDWFV	18	2	--	--	--
A6L-1-C9-IGFR	YRGMLVLGRISDGAGKVAFEPPARIGQNVFAVNFYDWFV	18	2	--	--	--
A6L-0-G10-IGFR	YRCMPVLGRISDGAG#VASDRPARIGQKVFAVNFYDWFV	18	2	--	--	--
A6L-1-G8-IGFR	YRGRLVLGRISDGAGKVAAEPPASMDSKVFAGNFYDWFV	15	2	--	--	--

FIG. 1H

Clone Design	Sequence GFREGNFYDWFVAQVT	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
E4Dα-1-B8-IR	GFREGQRWYWFVAQVT	40.7	1.0	12.3	0.1	12.3
E4Dα-3-E5-IR	GFREGYFYDWFLAQVT	39.6	2.0	1.5	1.3	0.8
E4Dα-1-A1-IR	GFREGDFYEWFVAQVT	48.7	44.9	31.4	1.4	0.7
E4Dα-2-D9-IR	GFREGQFYEWFAAQVT	22.9	3.3	2.4	1.4	0.7
E4Dα-1-B3-IR	GFREGTFYDWFVAQVT	41.8	38.6	26.5	1.5	0.7
E4Dα-1-A6-IR	GFREGNFYDWFEAQVT	56.3	51.2	32.6	1.6	0.6
E4Dα-1-A10-IR	GFREGAFYDWFEAQVT	48.9	42.2	26.5	1.6	0.6
E4Dα-1-A8-IR	GFREGAFYDWFVAQVT	46.9	41.5	26.2	1.6	0.6
E4Dα-1-B1-IR	GFREGKFYQWFEAQVT	44.1	31.1	19.7	1.6	0.6
E4Dα-2-C9-IR	GFREGDFYDWFQAQVT	34.0	8.1	4.8	1.7	0.6
E4Dα-1-A3-IR	GFREGTFYEWFVAQVT	45.3	40.3	22.5	1.8	0.6
E4Dα-1-A9-IR	GFREGNFYDWFVAQVT	46.9	41.0	22.5	1.8	0.5
E4Dα-3-F3-IR	GFREGQFYEWFLAQVT	37.2	14.1	8.0	1.8	0.6
E4Dα-2-D3-IR	GFREGQFYDWFLAQVT	35.1	16.3	8.7	1.9	0.5
E4Dα-2-D6-IR	GFREGEFYDWFQAQVT	33.2	5.6	2.8	2.0	0.5
E4Dα-3-F10-IR	GFREGQFYDWFRAQVT	27.8	4.5	2.3	2.0	0.5
E4Dα-2-D5-IR	GFREGYFYEWFQAQVT	43.8	23.8	11.4	2.1	0.5
E4Dα-3-F4-IR	GFREGDFYQWFEAQVT	25.9	7.6	3.7	2.1	0.5
E4Dα-3-E3-IR	GFREGSFYGWFQAQVT	34.6	4.0	1.9	2.1	0.5
E4Dα-3-F8-IR	GFREGSFYAWFQAQVT	20.9	16.0	7.4	2.2	0.5
E4Dα-2-C1-IR	GFREGQFYDWFVAQVT	43.1	11.6	5.0	2.3	0.4
E4Dα-1-B4-IR	GFREGIFYEWFVAQVT	45.3	6.6	2.9	2.3	0.4

FIG. 1I-1

Clone Design	Sequence GFREGNFYDWFVAQVT	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
E4Dα-4-H5-IR	GFREGSFYEWFQAQVT	47.2	36.0	14.7	2.4	0.4
E4Dα-1-B12-IR	GFREGNFYDWFAAQVT	47.6	33.4	13.8	2.4	0.4
E4Dα-4-G2-IR	GFREGSFYDWFVAQVT	23.4	20.4	8.6	2.4	0.4
E4Dα-3-F9-IR	GFREGDFYDWFVAQVT	36.2	15.6	6.3	2.5	0.4
E4Dα-4-G6-IR	GFREGDFYQWFVAQVT	26.0	4.9	2.0	2.5	0.4
E4Dα-4-H9-IR	GFREGGFYDWFVAQVT	47.8	24.8	9.5	2.6	0.4
E4Dα-2-C10-IR	GFREGDFYGWFQAQVT	42.4	23.2	9.0	2.6	0.4
E4Dα-1-B2-IR	GFREGVFYDWFVAQVT	39.4	18.7	7.2	2.6	0.4
E4Dα-3-F12-IR	GFREGGFYEWFQAQVT	38.9	16.6	5.6	3.0	0.3
E4Dα-2-D11-IR	GFREGSFYDWFQAQVT	40.2	11.1	3.3	3.4	0.3
E4Dα-4-H2-IR	GFREGNFYEWFQAQVT	37.8	33.9	8.2	4.1	0.2
E4Dβ-4-A12-IR	GFREGKFYDWFLAQVT	41.1	8.3	28.7	0.3	3.5
E4Dβ-4-A10-IR	GFREGEFYEWFVAQVT	5.8	1.2	2.4	0.5	2.0
E4Dβ-4-E10-IR	GFREGRFYDWFVAQVT	9.6	1.2	2.2	0.5	1.8
E4Dβ-4-B11-IR	GFREGTFYDWFVAQVT	36.1	15.2	26.9	0.6	1.8
E4Dβ-4-C10-IR	GFREGEFYEWFAAQVT	27.8	13.3	23.7	0.6	1.8
E4Dβ-4-E8-IR	GFREGDFYEWFEAQVT	28.7	16.7	28.2	0.6	1.7
E4Dβ-4-G7-IR	GFREGHFYDWF?AQVT	30.9	14.7	24.7	0.6	1.7
E4Dβ-4-C8-IR	GFREGEFYDWFVAQVT	35.5	22.5	32.9	0.7	1.5
E4Dβ-4-A8-IR	GFREGSFYDWFVAQVT	31.2	14.5	22.2	0.7	1.5
E4Dβ-4-A9-IR	GFREGSFYDWFGAQVT	35.8	9.0	13.1	0.7	1.5
E4Dβ-4-G11-IR	GFREGTFYDWFQAQVT	28.9	9.7	13.6	0.7	1.4
E4Dβ-4-B9-IR	GFREGNFYEWFTAQVT	27.2	9.1	12.5	0.7	1.4
E4Dβ-4-F10-IR	GFREGSFYNWFQAQVT	7.7	1.5	2.1	0.7	1.4
E4Dβ-4-D12-IR	GFREGNFYDWFVAQVT	41.1	27.2	36.1	0.8	1.3
E4Dβ-4-B8-IR	GFREGDFYDWFVAQVT	35.9	27.0	35.2	0.8	1.3
E4Dβ-4-G10-IR	GFREGAFYDWFAAQVT	38.5	25.5	33.7	0.8	1.3
E4Dβ-4-D9-IR	GFREGSFYDWFEAQVT	34.1	19.3	25.7	0.8	1.3

FIG. 11-2

Clone Design	Sequence GFREGNFYDWFVAQVT	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
E4Dβ-4-F8-IR	GFREGSFYDWFAAQVT	39.3	35.6	44.4	0.8	1.2
E4Dβ-4-E12-IR	GFREGSFYEWFDAQVT	40.2	27.8	33.4	0.8	1.2
E4Dβ-4-H12-IR	GFREGAFYDWFEAQVT	41.2	27.1	32.3	0.8	1.2
E4Dβ-4-C9-IR	GFREGQFYDWFAAQVT	38.0	22.5	27.6	0.8	1.2
E4D□-4-H9-IR	GFREGNFYDWFAAQVT	38.7	33.3	36.6	0.9	1.1
E4D□-4-G9-IR	GFREGDFYDWFAAQVT	10.9	4.9	5.6	0.9	1.1
E4Dβ-4-F12-IR	GFREGSFYEWFEAQVT	14.8	5.9	6.1	1.0	1.0
E4Dβ-4-F9-IR	GFREGGFYDWFLAQVT	39.3	31.3	28.3	1.1	0.9
E4Dβ-4-F7-IR	GFREGGFYAWFAAQVT	31.0	22.2	19.5	1.1	0.9
E4Dβ-4-B7-IR	GFREGGFYEWF?AQVT	--	--	--	--	--

FIG. 1I-3

Clone Design	Sequence GFREGNFYDWFVAQVT	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
E4D-2-E7-IGFR	GFREGDFYDWFRAQVT	20.8	22.8	--	--	--
E4D-2-C11-IGFR	GFREGSFYDWFVAQVT	21.5	22.6	--	--	--
E4D-2-B1-IGFR	GFREGDFYGWFQAQVT	22.0	22.5	--	--	--
E4D-2-D10-IGFR	GFREGGFYDWFQAQVT	20.6	22.1	--	--	--
E4D-2-A9-IGFR	GFREGDFYDWFVAQVT	17.4	21.5	--	--	--
E4D-2-E5-IGFR	GFREGDFYDWFQAQVT	24.2	21.2	--	--	--
E4D-2-H9-IGFR	GFREGGFYDWFVAQVT	19.1	20.7	--	--	--
E4D-1B-C4-IGFR	GFREGDFYDWFAAQVT	24.3	20.5	--	--	--
E4D-2-E10-IGFR	GFREGNFYDWFQAQVT	21.0	20.5	--	--	--
E4D-2-F4-IGFR	GFREGNFYDWFLAQVT	25.0	20.2	--	--	--
E4D-2-C10-IGFR	GFREGHFYDWFQAQVT	22.8	20.1	--	--	--
E4D-3-D8-IGFR	GFREGQFYEWFEAQVT	21.1	19.8	--	--	--
E4D-3-F9-IGFR	GFREGSFYEWFQAQVT	22.6	19.7	--	--	--
E4D-1B-E5-IGFR	GFREGDFYDWFLAQVT	24.2	18.8	--	--	--
E4D-2-F3-IGFR	GFREGHFYDWFVAQVT	23.6	18.0	--	--	--
E4D-3-D5-IGFR	GFREGQFYEWFVAQVT	22.2	18.0	--	--	--
E4D-3-G10-IGFR	GFREGQFYDWFAAQVT	22.1	17.6	--	--	--
E4D-2-F6-IGFR	GFREGQFYDWFVAQVT	24.6	17.5	--	--	--
E4D-2-F7-IGFR	GFREGDFYQWFAAQVT	19.0	17.5	--	--	--
E4D-3-B7-IGFR	GFREGNFYDWFVAQVT	23.0	16.4	--	--	--
E4D-1B-C12-IGFR	GFRDGSFYDWFVAQVT	23.0	16.1	--	--	--
E4D-3-B1-IGFR	GFREGHFYEWFQAQVT	21.6	16.0	--	--	--
E4D-2-E2-IGFR	GFREGDFYDWFSAQVT	21.9	14.1	--	--	--
E4D-2-D1-IGFR	GFREGHFYDWFDAQVT	24.5	13.2	--	--	--
E4D-1-D4-IGFR	GFREGYFYDWFKAQVT	18.9	12.4	--	--	--
E4D-1B-A10-IGFR	GFREGHFYDWFEAQVT	23.9	10.8	--	--	--
E4D-1B-A3-IGFR	GFREGDFYDWFEAQVT	22.2	10.8	--	--	--
E4D-1-B5-IGFR	GFREGTFYDWFVAQVT	19.0	10.8	--	--	--

FIG. 1J-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	GFREGNFYDWFVAQVT	--	--	--	--	--
E4D-1B-B8-IGFR	GFREGDYYGWFEAQVT	23.8	10.7	--	--	--
E4D-1-G7-IGFR	GFREGDFYAWFMAQVT	14.3	10.5	--	--	--
E4D-1B-A11-IGFR	GFREGNFYEWFLAQVT	24.0	10.0	--	--	--
E4D-1-C3-IGFR	GFREGSFYDWFDAQVT	15.8	9.3	--	--	--
E4D-2-H1-IGFR	GFREGNFYDQFVAQVT	19.6	4.9	--	--	--
E4D-1-C2-IGFR	GFREGHFYEWFAAQVT	11.5	4.5	--	--	--
E4D-1B-A12-IGFR	GFREGNFYEWFVAQVT	18.4	3.5	--	--	--
E4D-1B-A1-IGFR	GFREGKFYDWFVAQVT	22.5	2.9	--	--	--
E4D-2-A3-IGFR	GFREGMFDVQLLAQVT	22.7	2.1	--	--	--

FIG. 1J-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHENFYDWFVRQVSXXXXXX	--	--	--	--	--
Parental	VTFTSAVFHENFYDWFVRQVS	29.8	17.5	16.3	1.1	0.9
H2CA-4-F11-IR	TYKARFLHENFYDWFNRQVSQYFGRV	37.7	2.2	18.1	0.1	8.2
H2CA-4-E10-IR	QRLSLHEQFYDWFVGQVSPLGAGG	31.2	4.4	18.8	0.2	4.3
H2CA-4-G3-IR	GGGKVNFHEDFYGWFVQQFSGVGSDR	36.1	13.4	25.7	0.5	1.9
H2CA-3-A11-IR	LVGDAPFHEDFYDWFARQVFGCCQEQ	35.6	12.1	22.0	0.5	1.8
H2CA-4-F8-IR	TGAEVSFHENFYDWFDRQYSSWLDRD	36.0	21.1	33.5	0.6	1.6
H2CA-4-G4-IR	QPHSSRLHESFYDWFDRQVPWYALDR	37.1	23.3	34.3	0.7	1.5
H2CA-4-F4-IR	SRALAAVHEQFYDWFVRQVSGLDWGY	39.8	25.0	35.6	0.7	1.4
H2CA-4-H10-IR	QPKDGTLHENFYDWFVRQVSSSGWVG	33.5	5.1	6.6	0.8	1.3
H2CA-4-F1-IR	RGRLIQLHEDFYDWFLRQVSGMGGGS	36.1	19.6	25.1	0.8	1.3
H2CA-3-D5-IR	QRGAPKSDENFYDWFVRQVLRFGEND	39.3	24.3	31.9	0.8	1.3
H2CA-4-E11-IR	AARTSLFHEDFYEWFDRQVRQEGMWG	8.2	2.6	3.2	0.8	1.2
H2CA-3-B6-IR	GTSNHSLHENFYDWFVRQLSSVQSSG	35.9	9.9	12.1	0.8	1.2
H2CA-3-A9-IR	VSHVHLFHENFYDWFVRQLAAEGFSG	37.3	30.1	36.2	0.8	1.2
H2CA-4-H5-IR	GRQDSGLHEHFYDWFSRQVQGEVALG	38.6	35.4	37.3	1.0	1.1
H2CA-3-C9-IR	SNDERQFHETFYDWFVRQVSADGADR	29.3	5.1	5.6	0.9	1.1
H2CA-3-A10-IR	LSTEQRFHEKFYDWFVHQVSTSGGGT	37.2	16.9	19.1	0.9	1.1
H2CA-3-A3-IR	SLSREQFHENFYDWFARQVSELEGVV	29.2	28.6	32.2	0.9	1.1
H2CA-4-G8-IR	IPGRRSLHENFYDWFVRQVSPGGGSA	32.4	29.1	31.6	0.9	1.1
H2CA-4-G9-IR	TQKAQSLDEKFYDWFVRQVSGGGLTG	36.1	34.4	36.4	0.9	1.1
H2CA-4-G10-IR	VSQLSDFHENFYGWFARQIAGQAEWT	34.2	35.5	37.7	0.9	1.1
H2CA-4-H7-IR	NGTSQALHQNFYDWFAQQISGSEPGP	37.0	36.0	40.0	0.9	1.1
H2CA-4-F9-IR	VGQSVTFHGDFYDWFDRQLSGSQEFG	37.5	36.7	39.5	0.9	1.1
H2CA-4-F7-IR	TIDHHPLHEQFYDWFARQVSDLESLG	37.7	37.6	39.9	0.9	1.1
H2CA-3-D10-IR	PNVGYAFHENFYDWFIRQVSIEEKAG	18.7	3.6	3.5	1.0	1.0
H2CA-3-B1-IR	SRGSGVFHESFYNWFDRQVSEWIQFG	26.5	21.4	21.5	1.0	1.0
H2CA-3-A5-IR	QPVSGSVHERFYDWFVRQVSGSAGGG	32.9	22.9	22.4	1.0	1.0
H2CA-4-F10-IR	ASQLPPVYENFYEWFDRQVSLDAQRE	26.6	27.7	28.5	1.0	1.0

FIG. 1K-1

Clone Design	Sequence XXXXXXFHENFYDWFVRQVSXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
H2CA-3-D9-IR	VSGRGAFHENFYDWFVRQVFRDEQDT	36.6	30.6	30.9	1.0	1.0
H2CA-3-C2-IR	ARPPPTVHENFYDWFVRQVSETWRQD	38.3	30.7	31.0	1.0	1.0
H2CA-4-G1-IR	QGGDRLFHERFYDWFDRLVSSDSTGE	34.1	30.7	30.4	1.0	1.0
H2CA-4-E2-IR	QHIAAGLHENFYDWFIRQVSGVNVPA	33.9	31.0	31.8	1.0	1.0
H2CA-4-H9-IR	QPNDGLLHENFYDWFVRQVSNAVDGG	38.9	31.1	31.4	1.0	1.0
H2CA-3-D2-IR	PVEFTVYHDNFYDWFARQVSDGLGQF	33.0	31.1	29.8	1.0	1.0
H2CA-3-B3-IR	FCVQASIHENFYDWFVRQVAENQVFS	35.3	31.4	30.0	1.0	1.0
H2CA-4-G11-IR	GRPRGSFHENFYDWFARQVSGDGAGT	37.9	31.9	31.0	1.0	1.0
H2CA-4-F2-IR	IVGASLCHESFYDWFACQVTNLQSQG	38.1	32.0	31.9	1.0	1.0
H2CA-3-C5-IR	IGLRQMFHENFYDWFAREVSKEAGDG	36.9	32.3	31.6	1.0	1.0
H2CA-3-B2-IR	LGGAIEGHGNFYDWFVRQVSLDVGGE	36.6	32.7	32.5	1.0	1.0
H2CA-3-B11-IR	LNALQQLHENFYDWFGRQVSATPPGG	35.5	32.8	33.3	1.0	1.0
H2CA-4-G2-IR	VGNCDTFPENFYDWFACQVSELGGMN	35.9	33.0	33.4	1.0	1.0
H2CA-3-A4-IR	FSQDGNFHENFYDWFDRQLSLVGAGT	33.3	33.0	32.9	1.0	1.0
H2CA-4-H3-IR	PAGNRALHESFYDWFVRQVSEFQLGA	39.5	33.7	33.7	1.0	1.0
H2CA-4-G5-IR	DRLRARFNENFYDWFDRQVSGQGSMP	35.3	34.0	35.6	1.0	1.0
H2CA-4-E8-IR	VLGVAQFHDKFYDWFARQVSQLESAG	35.7	34.7	34.9	1.0	1.0
H2CA-4-G6-IR	GVVGGAFHEQFYDWFDRQVSAAFKGD	36.2	35.0	33.5	1.0	1.0
H2CA-3-B7-IR	DESEMRLHEQFYDWFARLVSLEGGSA	37.6	36.5	35.3	1.0	1.0
H2CA-3-B4-IR	EGGGVAIHENFYDWFDRQVSLQGWSD	39.8	36.5	35.1	1.0	1.0
H2CA-3-C7-IR	SRIVSRFHENFYDWFVRQVSGDAPVQ	40.2	36.7	35.9	1.0	1.0
H2CA-4-E5-IR	IPAGAQLHENFYDWFARQVSGEDGGA	37.3	37.0	36.3	1.0	1.0
H2CA-4-E7-IR	GSSAAGFDEQFYDWFDRQVSEAFRDG	39.7	37.6	37.6	1.0	1.0
H2CA-3-B9-IR	RLALRTFHQDFYDWFVRQVAAEDTDP	39.4	37.7	37.6	1.0	1.0
H2CA-4-F5-IR	QGSFAVLHENFYDWFARQVSGVEGLA	38.8	38.0	37.8	1.0	1.0
H2CA-3-B10-IR	QGNMSALHENFYDWFVRQVSEADRVD	41.9	38.9	38.0	1.0	1.0
H2CA-3-A12-IR	VAYPALLHEQFYDWFVRQVSAVAGTT	37.8	7.3	6.3	1.2	0.9
H2CA-3-A8-IR	PDTINSQHKNFYDWFVRQVSGVGTSS	36.8	22.5	19.2	1.2	0.9

FIG. 1K-2

Clone Design	Sequence XXXXXXFHENFYDWFVRQVSXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
H2CA-3-D12-IR	SEDVDSRHENFYDWFVRQVSGIGLQD	36.8	34.1	29.6	1.2	0.9
H2CA-3-B5-IR	PAPADAFDHNFYDWFARQLSATTTIQ	38.8	35.2	30.5	1.2	0.9
H2CA-4-E1-IR	MVQRISIHENFYDWFVRQISGSAVPP	29.8	12.5	11.3	1.1	0.9
H2CA-3-D3-IR	GNVRGQFHGQFYDWFARQVSGSEGDA	33.1	29.9	27.5	1.1	0.9
H2CA-4-E3-IR	PDAEKQFHETFYGWFVRQISEDSANS	33.3	32.3	30.2	1.1	0.9
H2CA-4-E12-IR	FGRGVHCDENFYDWFVCQVSGALLEG	36.0	32.4	29.4	1.1	0.9
H2CA-3-A6-IR	ETPLTELHEQFYDWFVRQVSGFPGGV	34.0	33.1	30.6	1.1	0.9
H2CA-4-E9-IR	QHRGPHFHEDFYDWFVRQVSSAVPSD	38.8	33.7	29.7	1.1	0.9
H2CA-4-F3-IR	RQDPGLFHDNFYDWFDRLVSAWDGQE	41.0	34.2	32.0	1.1	0.9
H2CA-4-H6-IR	QAAVGVCNKDFYAWFACQVREDFAKA	37.1	34.5	30.8	1.1	0.9
H2CA-4-H2-IR	RNWNLQFNENFYDWFDRQVSALRGGG	41.8	35.3	32.8	1.1	0.9
H2CA-3-D4-IR	RSEQYRFHENFYEWFDRQVSRMGLLG	38.7	35.5	32.3	1.1	0.9
H2CA-3-D1-IR	GAGGRDFDEDFYDWFVRQVSGQVTSG	34.5	35.5	31.3	1.1	0.9
H2CA-3-C1-IR	SPEGNLVHDQFYDWFVRQLSSTSAGT	39.9	36.1	32.9	1.1	0.9
H2CA-3-D8-IR	QGGLGDFDEDFYDWFARQVSRRDRAD	37.8	36.7	33.1	1.1	0.9
H2CA-4-H4-IR	LSQGVGFQENFYEWFERQVSGWDGRD	38.5	37.0	33.7	1.1	0.9
H2CA-4-F6-IR	VFERSRCHDNFYDWFFCQVSGQADGG	38.7	37.5	35.2	1.1	0.9
H2CA-4-E4-IR	LLASRAFHENFYDWFARQVSGTQPPG	38.6	38.0	34.7	1.1	0.9
H2CA-3-C11-IR	VPDAQIFHESFYDWFVRQASAGGPAD	40.3	38.3	36.1	1.1	0.9
H2CA-3-C4-IR	ANQMGRFHDNFYDWFDRQVSRYERGT	41.9	38.4	35.0	1.1	0.9
H2CA-4-E6-IR	PSRKDGLHQSFYDWFARQVQDMEGRA	39.3	38.8	35.8	1.1	0.9
H2CA-3-D7-IR	QAVTRRFHENFYDWFARQVSEEGGWS	42.5	39.2	35.5	1.1	0.9
H2CA-3-A7-IR	GYAVGQYQANFYDWFVRQVDGMSNGG	35.3	15.2	11.6	1.3	0.8
H2CA-4-G12-IR	GHQRDLLHESFYDWFVRQVSEAEGGG	37.6	19.4	15.1	1.3	0.8
H2CA-3-D6-IR	DRPSSFIHENFYEWFARQVSQSGSSG	39.4	36.2	27.6	1.3	0.8
H2CA-4-H12-IR	ERTAETLHEQFYDWFVRQVSAMDGES	40.0	38.4	29.3	1.3	0.8
H2CA-3-D11-IR	LTSQLLSHEDFYDWFVRQVSGVGGSG	38.1	32.9	27.2	1.2	0.8
H2CA-3-C12-IR	PDRSDRLDDNFYDWFVRQVSQVINED	38.5	38.4	31.7	1.2	0.8

FIG. 1K-3

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHENFYDWFVRQVSXXXXXX	--	--	--	--	--
H2CA-4-G7-IR	RAGGVGLHDNFYDWFVRQVSGGDSGP	35.9	34.7	23.7	1.5	0.7
H2CA-3-C6-IR	ADCYVQLHENFYDWFRRQVCNLQEGM	38.7	37.6	28.2	1.3	0.7
H2CA-3-B8-IR	RQGHAGFHDNFYDWFVRQVSGSTPQV	37.8	19.6	9.9	2.0	0.5

FIG. 1K-4

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHENFYDWFVRQVSXXXXXX	--	--	--	--	--
Parental	VTFTSAVFHENFYDWFVRQVS	29.8	17.5	16.3	1.1	0.9
H2CA-4-G9-IGFR	GIISQSCPESFYDWFAGQVSDPWWCW	8.6	9.5	0.6	16.0	0.1
H2CA-4-H6-IGFR	VGRASGFPENFYDWFGRQLSLQSGEQ	4.9	10.5	0.7	14.6	0.1
H2CA-4-F-IGFR5	VGYQGQGDENFYDWFIRQVSGRLGVQ	5.5	9.7	0.8	12.3	0.1
H2CA-4-H8-IGFR	SACQFDCHENFYDWFARQVSGGAAYG	5.6	9.2	1.0	9.4	0.1
H2CA-4-F11-IGFR	SAAQLFFQESFYDWFLRQVAESSQPN	3.5	6.8	1.0	6.7	0.1
H2CA-4-F6-IGFR	AVRATRFDEAFYDWFVRQISDGQGNK	3.9	7.3	1.1	6.4	0.2
H2CA-4-F10-IGFR	VNQSGSIHENFYDWFERQVSHQRGVR	4.9	5.7	1.0	5.9	0.2
H2CA-1-A3-IGFR	APDPSDFQEIFYDWFVRQVSRMPGGG	7.7	3.8	0.8	5.1	0.2
H2CA-3-C8-IGFR	SSCDGAGHESFYEWFVRQVSGCRSV	15.1	5.6	1.2	4.8	0.2
H2CA-2-B9-IGFR	RAGSSDFHEDFYEWFVRQVSLSLKGK	9.3	7.0	1.7	4.2	0.2
H2CA-4-H4-IGFR	QAVQPGFHEEFYDWFVRQVSTGVGGG	3.9	4.1	1.0	4.2	0.2
H2CA-4-F7-IGFR	SSIGGGFHENFYDWFSRQLSQSPPLK	1.5	3.2	0.8	4.1	0.2
H2CA-3-D6-IGFR	QSPVGSSHEDFYDWFFRQVAQSGAHQ	8.3	9.0	2.2	4.0	0.3
H2CA-3-D8-IGFR	NYRRQVFNGNFYDWFDRQVFSLVTPG	10.9	7.2	1.8	4.0	0.3
H2CA-4-G11-IGFR	TLDGGSFEEQFYDWFVRQLSYRTNPD	10.8	9.5	2.5	3.9	0.3
H2CA-4-F1-IGFR	FYVQQWGHENFYDWFDRQVSQSGGAG	5.8	3.5	0.9	3.8	0.3
H2CA-3-D7-IGFR	LRRQAPVEENFYDWFVRQVSGDRVGG	13.3	3.0	0.8	3.7	0.3
H2CA-1-A7-IGFR	RCGRELYHSTFYDWFDRQVAGRTCPS	8.0	2.2	0.6	3.7	0.3
H2CA-2-B4-IGFR	CCLLCRFQQNFYDWFVCQGISRLRPL	3.5	4.1	1.1	3.6	0.3
H2CA-2-B3-IGFR	PPLASDLDVQFYGWFVQQVSPPGRGG	7.7	3.8	1.0	3.6	0.3
H2CA-2-B2-IGFR	GAPVDQLHEDFYDWFVRQVSQAATG	4.1	3.4	1.0	3.5	0.3
H2CA-3-D4-IGFR	RSASGSLPEQFYDWFVRQVSLSGTDK	17.6	13.8	4.1	3.4	0.3
H2CA-4-F2-IGFR	SRVTTVFHENFYDWFVRQLSDSAISG	9.3	12.8	4.2	3.0	0.3
H2CA-3-D11-IGFR	DERGGKFREDFYDWFVRQVSESRFGQ	12.2	6.9	2.3	3.0	0.3
H2CA-4-H9-IGFR	RGAVAGFHDQFYDWFDRQVSRVHKFG	8.7	5.6	1.9	3.0	0.3
H2CA-2-B11-IGFR	AICDAGFHEHFYDWFALQVSDCGRQS	11.9	4.6	1.6	3.0	0.3
H2CA-3-E8-IGFR	LGYQEPFQQNFYDWFVRQVSGAENAG	13.2	6.3	2.2	2.9	0.3

FIG. 1L-1

Clone Design	Sequence XXXXXXFHENFYDWFVRQVSXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
H2CA-3-E6-IGFR	WRGHGTFHEDFYDWFVRQVSGSGSST	15.7	8.7	3.1	2.8	0.4
H2CA-4-F4-IGFR	GGRVGVLHENFYDWFDRQVSLRGADG	11.5	7.4	3.0	2.5	0.4
H2CA-3-D10-IGFR	CNLTAGFHEQFYHWFAIQVCGDAENA	9.4	6.8	2.9	2.3	0.4
H2CA-3-E1-IGFR	ERGEDMFHENFYDWFVRQISGRQGGG	12.5	6.4	2.8	2.3	0.4
H2CA-2-B6-IGFR	TNQGVGFYDSFYGWFVRQIQYGVDSG	18.0	6.2	2.7	2.3	0.4
H2CA-3-E11-IGFR	HLADGQFHEKFYDWFERQISSRCNDC	4.7	2.2	1.0	2.2	0.5
H2CA-4-H2-IGFR	QTFGKSLHENFYDWFVRQVSREEGGD	9.8	9.9	4.8	2.1	0.5
H2CA-3-C11-IGFR	FRTLAAQHDSFYDWFDRQVSGAAGER	9.3	3.3	1.6	2.1	0.5
H2CA-2-B8-IGFR	SASTHQFHENFYDWFVRQVSGAQKIL	14.6	7.9	3.9	2.0	0.5

FIG. 1L-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHXXFYXWFXXXXXX	--	--	--	--	--
Parental	VTFTSAVFHENFYDWFVRQVS	29.8	17.5	16.3	1.1	0.9
H2CBα-3-B12-IR	QSDSGTVHDRFYGWFRDT*A	26.0	1.3	20.4	0.1	16.0
H2CBα-3-D2-IR	WTDVDGFHSGFYRWFQNQWER	20.6	1.7	12.1	0.1	7.0
H2CBα-3-D12-IR	VASGHVLHGQFYRWFVDQFAL	24.6	2.1	14.0	0.1	6.7
H2CBα-3-H5-IR	QARVGNVHQQFYEWFREVMQG	16.7	2.4	15.1	0.2	6.3
H2CBα-3-B6-IR	VGDFCVSHDCFYGWFLRESMQ	31.4	2.5	13.9	0.2	5.6
H2CBα-3-G11-IR	SGSRPVFHEQFYEWFVDQLG	22.7	1.4	6.4	0.2	4.7
H2CBα-3-A6-IR	QFSAGAFHGDFYGWFRALYNG	25.9	1.7	7.1	0.2	4.3
H2CBα-3-B1-IR	SRFDERLHHQFYEWFRVLNEP	33.4	6.0	25.5	0.2	4.3
H2CBα-3-F8-IR	DSVNSDLHRAFYGWFAEQWRA	23.0	4.8	19.8	0.2	4.1
H2CBα-3-E11-IR	GSVDREIHGPFYSWFSEQLWG	14.0	2.2	8.5	0.3	4.0
H2CBα-3-G4-IR	SAKTPVLHDGFYMWFEAQSES	24.9	2.2	6.9	0.3	3.2
H2CBα-3-D3-IR	LVVGRRFHQSFYDWFVAAAGG	23.6	2.6	8.0	0.3	3.1
H2CBα-3-C1-IR	IMWPCTFQDPFYCWFQTEQGR	27.0	5.6	16.4	0.3	2.9
H2CBα-3-C3-IR	VVGPLDIHERFYGWFHQQGGA	23.3	1.1	3.1	0.4	2.8
H2CBα-3-G3-IR	VVPKAGFHEAFYEWFRRQDRD	23.7	6.7	17.6	0.4	2.6
H2CBα-3-E4-IR	QSFVTSVHTRFYAWFASALEM	28.8	8.3	21.9	0.4	2.6
H2CBα-3-G5-IR	SRGLGLYHSGFYGWFERQFNQ	26.7	7.0	17.2	0.4	2.5
H2CBα-3-B11-IR	GADTGAVHRRFYLWFEQLSGG	28.0	8.6	19.4	0.4	2.3
H2CBα-3-A1-IR	PGNRPTFHAEFYRWFREAQGS	31.3	11.3	24.9	0.5	2.2
H2CBα-3-H1-IR	VAVAWGLHESFYAWFENQFSD	27.2	10.6	23.9	0.4	2.2
H2CBα-3-F12-IR	GFNTGTFHDQFYYWFWEAAGG	21.1	6.1	12.7	0.5	2.1
H2CBα-3-H7-IR	GDGLTAFHQGFYEWFDIQMYG	21.0	9.7	19.1	0.5	2.0
H2CBα-3-C12-IR	VGVNRQFHTRFYAWFDEQLGG	26.0	12.7	24.7	0.5	1.9

FIG. 1M-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHXXFYXWFXXXXXX	--	--	--	--	--
H2CBα-3-D11-IR	GPRGQRLHDAFYSWFDALRVN	27.8	13.0	24.8	0.5	1.9
H2CBα-3-H12-IR	LGTLAVFHELFYGWFERQLGG	27.4	7.2	12.4	0.6	1.7
H2CBα-3-A10-IR	LGGYCGFNCQFYRWFDNLADR	27.1	13.2	22.3	0.6	1.7
H2CBα-3-A5-IR	FSGWADYQSGFYQWFAEELAN	28.3	16.1	28.1	0.6	1.7
H2CBα-3-C4-IR	WGPFSVFDESFYRWFAQASDD	30.7	17.2	29.2	0.6	1.7
H2CBα-3-B8-IR	PRNEGLVHGLFYDWFQRALSG	25.6	11.3	18.6	0.6	1.6
H2CBα-3-H11-IR	DEGGAPLDVMFYRWFEQAVRG	28.8	14.0	22.4	0.6	1.6
H2CBα-3-E10-IR	QSGNRGSHGAFYSWFRDVLAN	27.7	14.3	23.0	0.6	1.6
H2CBα-3-C2-IR	MRQRDGFNSSFYGWFAAALGE	28.4	17.0	26.7	0.6	1.6
H2CBα-3-F6-IR	SEERKKVHSQFYSWFDRQLLG	27.3	14.5	21.8	0.7	1.5
H2CBα-3-D4-IR	PSPNAPFHGGFYDWFDWVQGS	29.0	18.9	27.1	0.7	1.4
H2CBα-3-A7-IR	FHRPGSFNTNFYQWFDDQMNQ	29.1	19.4	26.9	0.7	1.4
H2CBα-3-H4-IR	SDDSSTLNGRFYTWFHMQLLD	27.2	20.1	27.9	0.7	1.4
H2CBα-3-B7-IR	QRGGGGFHEGFYSWFRSQSLL	28.6	18.0	23.6	0.8	1.3
H2CBα-3-F9-IR	SGSRPVFHEQFYEWFVDQLGL	26.1	19.1	24.3	0.8	1.3
H2CBα-3-H6-IR	GGSSQAFHGAFYEWFSAQLRG	24.8	21.6	27.3	0.8	1.3
H2CBα-3-F5-IR	AFVSERVNQRFYDWFRDQMRS	29.4	22.0	27.8	0.8	1.3
H2CBα-3-A2-IR	VRHPTRFHDEFYRWFTEQLTT	30.7	22.5	29.1	0.8	1.3
H2CBα-3-F3-IR	ARLLNIFDRGFYNWFQRQLDE	16.3	6.7	9.0	0.7	1.3
H2CBα-3-G6-IR	PSLSSNLHESFYRWFDQLVST	24.9	21.0	24.4	0.9	1.2
H2CBα-3-G7-IR	FAFGLGFHQGFYDWFAHQLEG	24.4	18.7	23.0	0.8	1.2
H2CBα-3-C5-IR	VSATVMLHREFYDWFGLQLLD	26.4	21.2	25.4	0.8	1.2
H2CBα-3-G1-IR	GGVSGVLHDRFYSWFERQLAG	26.9	21.5	26.3	0.8	1.2
H2CBα-3-E3-IR	GLGIASFHEGFYSWFTAQLGA	24.2	17.2	19.3	0.9	1.1

FIG. 1M-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHXXFYXWFXXXXXX	--	--	--	--	--
H2CBα-3-A9-IR	RVDAAALNAGFYEWFRGVIQG	30.5	21.7	24.1	0.9	1.1
H2CBα-3-C11-IR	GGAGRSFHDAFYEWFERQMAG	26.4	21.8	23.2	0.9	1.1
H2CBα-3-B4-IR	EGARQGFHARFYSWFAQQLAL	30.9	22.0	24.3	0.9	1.1
H2CBα-3-F11-IR	VLLPGVVHGGFYDWFSRQLSS	24.5	22.5	23.9	0.9	1.1
H2CBα-3-G10-IR	GALSDRYNNVFYDWFREQLLG	28.3	23.6	27.1	0.9	1.1
H2CBα-3-D7-IR	PDSFMSLHQRFYSWFQAQVGT	31.4	23.6	25.3	0.9	1.1
H2CBα-3-E2-IR	RVYKANFHNEFYGWFREQLLG	26.8	24.0	25.7	0.9	1.1
H2CBα-3-B5-IR	HSGMRDVHARFYSWFSEQLSG	28.7	25.0	26.4	0.9	1.1
H2CBα-3-C7-IR	ARLLERFQDPFYEWFETLMGD	30.0	25.2	28.7	0.9	1.1
H2CBα-3-G9-IR	RNSSGNFHDKFYNWFEAQLKG	27.8	25.2	26.7	0.9	1.1
H2CBα-3-A12-IR	GSMSPVFNDQFYGWFRDLVDE	28.0	26.4	28.7	0.9	1.1
H2CBα-3-C9-IR	SCTGRQFDGCFYAWFEDQLVG	32.1	28.7	31.9	0.9	1.1
H2CBα-3-B10-IR	GIAVQSLHDSFYRWFDNALGS	33.5	30.8	33.2	0.9	1.1
H2CBα-3-E1-IR	IGPPGSLHRGFYDWFAEQVEA	31.7	30.5	29.0	1.1	1.0
H2CBα-3-G12-IR	GAAGISFHRGFYDWFAAQVRD	29.1	31.4	29.8	1.1	1.0
H2CBα-3-F7-IR	GVDVTDFHKDFYSWFQRQLNG	23.2	20.7	20.3	1.0	1.0
H2CBα-3-G8-IR	WAGRAGIHGGFYEWFNRQLRG	22.8	20.9	20.4	1.0	1.0
H2CBα-3-C6-IR	LGQLAAFHLGFYEWFSEAVAA	26.7	21.2	22.0	1.0	1.0
H2CBα-3-H9-IR	VHSVSRLNVGFYQWFQDQLSG	23.4	22.5	22.0	1.0	1.0
H2CBα-3-H8-IR	LGLMAIFDRGFYGWFEQQLSG	23.5	23.4	23.2	1.0	1.0
H2CBα-3-F2-IR	VARGSSLHDDFYEWFASQLRT	25.5	24.3	25.2	1.0	1.0
H2CBα-3-D5-IR	LGYIGALNTQFYSWFADLVGS	26.7	24.5	25.6	1.0	1.0
H2CBα-3-D10-IR	EDSRLRLHEGFYGWFRKQLGD	26.8	24.9	24.9	1.0	1.0
H2CBα-3-F10-IR	GRDNMKFHSGFYDWFTQQLAG	25.7	25.6	26.1	1.0	1.0

FIG. 1M-3

Clone Design	Sequence XXXXXXFHXXFYXWFXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
H2CBα-3-D6-IR	AGVMGGFHQEFYLWFERALSN	27.9	26.0	25.8	1.0	1.0
H2CBα-3-H3-IR	AGHVGQVYDGFYGWFREQLGA	27.0	26.9	26.2	1.0	1.0
H2CBα-3-F4-IR	FVQNIGFDYDFYGWFVREVEK	31.2	27.2	27.7	1.0	1.0
H2CBα-3-E9-IR	PVGIGGLHRAFYQWFQSQVDA	31.6	27.7	28.2	1.0	1.0
H2CBα-3-H10-IR	GSRQEADHQAFYDWFNLVLGV	26.9	27.9	28.8	1.0	1.0
H2CBα-3-G2-IR	AGGRKPFHDDFYGWFRDQLAE	29.1	28.1	28.8	1.0	1.0
H2CBα-3-B2-IR	DLASHGFHDAFYNWFSVQLNS	29.4	28.1	28.2	1.0	1.0
H2CBα-3-E8-IR	GSNGGGVHGQFYAWFVEALSG	31.5	28.4	29.1	1.0	1.0
H2CBα-3-E5-IR	RGRASTFHDGFYGWFSQQLRF	33.0	28.7	28.9	1.0	1.0
H2CBα-3-E6-IR	SPARRVSHHDFYGWFAKQLES	29.6	29.0	28.1	1.0	1.0
H2CBα-3-E7-IR	SSDVGAFHSAFYDWFKAQLSG	30.4	30.2	30.2	1.0	1.0
H2CBα-3-C8-IR	PTVHRAFDDLFYGWFAKQVED	31.9	31.2	31.5	1.0	1.0
H2CBα-3-A4-IR	SSNTVGLDERFYAWFVDQLGA	32.2	31.9	32.6	1.0	1.0
H2CBα-3-D1-IR	PGAAEGFHSAFYDWFAQAVSG	32.9	32.5	31.5	1.0	1.0
H2CBα-3-B9-IR	MRSEASFHVEFYSWFEEQLRS	33.2	33.8	33.3	1.0	1.0
H2CBα-3-D8-IR	VSRYGGQQDGFYHWFSDLLKG	26.3	20.2	19.1	1.1	0.9
H2CBα-3-F1-IR	RPSSGGLHYGFYHWFRVQEEM	28.8	28.0	26.4	1.1	0.9
H2CBα-3-A11-IR	SNIEEHFHMQFYRWFSDALGN	20.5	21.5	17.7	1.2	0.8
H2CBα-3-A3-IR	ANDCLGLHAGFYGWFACQLGG	30.4	29.6	21.8	1.4	0.7

FIG. 1M-4

Clone Design	Sequence XXXXXXFHXXFYXWFXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
H2CBβ-3-E8-IR	TGHRLGLDEQFYWWFRDALSG	15.9	1.9	11.8	0.2	6.1
H2CBβ-4-F8-IR	VLTSNTLHQRFYSWFAAARRE	13.4	0.8	2.6	0.3	3.4
H2CBβ-3-C4-IR	CVAQGGFQSSFYCWFAGLDID	21.1	1.3	4.0	0.3	3.1
H2CBβ-3-D5-IR	NGQSSRFHTAFYDWFAAQLSG	14.0	3.3	10.2	0.3	3.1
H2CBβ-3-E6-IR	SVPRGTVHDAFYQWFREVALG	5.7	0.7	2.1	0.3	3.1
H2CBβ-4-G12-IR	GARGSTFHDQFYEWFWVQLGD	6.8	1.8	5.4	0.3	3.1
H2CBβ-4-F4-IR	PPGMNGFHTSFYSWFVDQLGD	17.9	1.9	5.6	0.3	3.0
H2CBβ-4-F11-IR	AVGTLGYHSGFYRWFERQLGG	15.0	1.7	4.8	0.3	2.9
H2CBβ-3-E5-IR	ELQARGVHRNFYRWFEAQVSG	17.0	1.8	5.0	0.4	2.8
H2CBβ-4-F2-IR	HRVARAFHEQFYDWFEKAVSG	15.9	1.3	3.4	0.4	2.6
H2CBβ-4-G4-IR	GAMEPDYHRSFYQWFAAALGE	8.7	1.4	3.5	0.4	2.6
H2CBβ-3-C8-IR	CPDRQSVDDRFYNWFADALAS	4.9	1.4	3.2	0.4	2.3
H2CBβ-4-F10-IR	GGAQISFHERFYQWFLQEAAG	10.2	1.0	2.4	0.4	2.3
H2CBβ-4-H4-IR	HKRGIVQHGAFYAWFDSLLSG	20.8	4.2	9.5	0.4	2.3
H2CBβ-4-G6-IR	QASDNRSDGQFYLWFEKLLSS	14.5	5.6	8.5	0.7	1.5
H2CBβ-4-H1-IR	DRGRMGVDEGFYNWFARQMQE	17.0	10.1	13.2	0.8	1.3

FIG. 1M-5

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHXXFYXWFXXXXXX	--	--	--	--	--
Parental	VTFTSAVFHENFYDWFVRQVS	**29.8**	**17.5**	**16.3**	**1.1**	**0.9**
H2CB-3-D2-IGFR	TASQECFDDGFYGWFRAWRCT	22.9	18.6	11.8	1.6	0.6
H2CB-3-C12-IGFR	SLDWRWSEEPFYRWFQRALAG	17.3	19.6	13.0	1.5	0.7
H2CB-3-B11-IGFR	CMSLSDCHRKFYGWFKSQGGE	24.6	17.1	11.9	1.4	0.7
H2CB-4-E2-IGFR	LALCRRSPGSFYGWFQAAVGC	22.4	21.0	16.5	1.3	0.8
H2CB-3-A5-IGFR	PRSATMSDGGFYWWFASQLGL	28.8	26.1	22.6	1.2	0.9
H2CB-4-G12-IGFR	LRRSSVFHDPFYE*ISRLVGG	23.7	23.8	19.4	1.2	0.8
H2CB-3-B2-IGFR	ARLQQQFHGGFYEWFRAQVSP	23.0	19.9	16.4	1.2	0.8
H2CB-3-D1-IGFR	AQLDNLCHEPFYSWFCAVTRE	21.5	19.5	15.7	1.2	0.8
H2CB-3-B6-IGFR	WTCDTAFHQDFYQWFCDKLGV	16.3	4.5	3.7	1.2	0.8
H2CB-4-F7-IGFR	GKEGFGLDRDFYWWFREQLGP	22.0	19.0	18.0	1.1	0.9
H2CB-4-G8-IGFR	GRAPSSFDCDFYCWFRNQVQS	20.2	18.6	16.5	1.1	0.9
H2CB-3-D4-IGFR	DVEAETQHRLFYAWFLSQLGS	21.9	18.3	16.9	1.1	0.9
H2CB-3-D5-IGFR	ISVTAVFHDGFYGWFNEQVSK	21.4	17.9	16.4	1.1	0.9
H2CB-4-E6-IGFR	NSEHGRLDVDFYGWFARVIQQ	19.6	15.8	14.8	1.1	0.9
H2CB-3-C2-IGFR	GPLGDGCQDGFYGWFMCQVST	18.8	12.2	10.8	1.1	0.9
H2CB-3-A6-IGFR	KRSAYNFHDPFYDWFRMQLSG	26.8	29.0	28.1	1.0	1.0
H2CB-4-H12-IGFR	ASEPGGYLDPFYGWFREQLRA	23.9	28.3	28.1	1.0	1.0
H2CB-3-B10-IGFR	NRGDGGVHSGFYNWFRLQLSG	27.1	27.5	27.3	1.0	1.0
H2CB-4-F11-IGFR	ASKGSSLHNDFYGWFAQQLAR	25.5	25.5	24.6	1.0	1.0
H2CB-4-G11-IGFR	ANVSMWIQVGFYDWFDAQLRQ	25.3	25.4	25.3	1.0	1.0
H2CB-4-E12-IGFR	RTSPGSLHDPFYDWFQQQLGG	27.8	24.9	24.7	1.0	1.0
H2CB-4-G10-IGFR	PGVMSSFHGGFYSWFREQLNG	25.1	24.6	24.2	1.0	1.0
H2CB-3-B9-IGFR	CLANSEDHDSFYGWFCQALGG	25.6	23.3	23.7	1.0	1.0
H2CB-3-B7-IGFR	GGSMGGMHGSFYEWFALQLRS	24.0	23.2	23.5	1.0	1.0
H2CB-4-H4-IGFR	RPQGGSIHAGFYQWFRDAVAG	23.5	23.1	23.8	1.0	1.0

FIG. 1N-1

Clone Design	Sequence XXXXXXFHXXFYXWFXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
H2CB-4-H10-IGFR	GALSSLFDAAFYDWFNRQLEG	21.9	22.4	23.3	1.0	1.0
H2CB-4-H5-IGFR	KVDLRGFHDGFYGWFARQLAG	22.3	22.3	21.6	1.0	1.0
H2CB-4-G7-IGFR	CSGLQRCHDSFYSWFESVVRE	23.1	21.6	20.6	1.0	1.0
H2CB-4-F4-IGFR	DSLGISFHEGFYDWFRRQLDM	21.3	20.9	21.3	1.0	1.0
H2CB-3-D8-IGFR	SGVFNGTFYDWFRIQLGE	20.0	20.5	21.6	1.0	1.0
H2CB-4-E4-IGFR	GYREMRSDLGFYQWFRDQLGL	21.6	20.5	21.2	1.0	1.0
H2CB-4-E5-IGFR	SVFMQHDHVGFYAWFRSLMEE	22.0	19.9	20.9	1.0	1.0
H2CB-4-E8-IGFR	FRHITEVDRSFYGWFVEQLRG	21.1	19.7	20.7	1.0	1.1
H2CB-3-D12-IGFR	WAGGSDVDGSFYDWFQRLLAS	26.6	17.3	16.8	1.0	1.0
H2CB-4-G9-IGFR	GLQNVSFHSGFYEWFARQVSQ	21.6	14.5	15.2	1.0	1.1
H2CB-3-C8-IGFR	SRVSDPYHVGFYQWFEEVVRG	20.8	13.4	13.9	1.0	1.0
H2CB-3-A12-IGFR	MGGATFFHTGFYDWFAAQLQH	28.6	27.5	29.2	0.9	1.1
H2CB-3-B12-IGFR	RPASRPFHSGFYQWFADQLSH	27.8	25.2	27.1	0.9	1.1
H2CB-3-A9-IGFR	GLAPGNFHEDFYRWFQEQTLG	27.7	24.3	25.7	0.9	1.1
H2CB-3-A3-IGFR	TAAISDFNSLFYGWFEQLLSS	26.9	24.1	26.5	0.9	1.1
H2CB-3-B4-IGFR	LDEDLPQHAGFYGWFAEALGV	25.8	23.8	25.3	0.9	1.1
H2CB-4-E7-IGFR	ASHKSAFDDNFYRWFSMQLRD	24.6	21.6	24.0	0.9	1.1
H2CB-4-G6-IGFR	HTGAGDLHGAFYNWFLEQLGG	22.4	21.1	23.0	0.9	1.1
H2CB-4-E9-IGFR	RRGRDGFHGGFYDWFAAQLSD	24.3	20.7	22.0	0.9	1.1
H2CB-4-H2-IGFR	GNFREAFHADFYSWFERQLQS	21.6	20.2	21.9	0.9	1.1
H2CB-3-A10-IGFR	RDTLPAFHQHFYQWFEKQVSA	24.3	19.9	21.5	0.9	1.1
H2CB-3-C4-IGFR	ERETAAFGQAFYQWFRDQIAG	23.1	19.2	22.0	0.9	1.1
H2CB-3-B5-IGFR	WGEGGGFYDWFYDQLGWEPSH	24.2	18.8	20.7	0.9	1.1
H2CB-4-G4-IGFR	SLVAADLHEGFYGWFRSQLGG	21.7	18.7	21.2	0.9	1.1
H2CB-3-D9-IGFR	TSEVGDFHAEFYSWFEIQLGR	24.4	18.6	20.0	0.9	1.1
H2CB-3-C3-IGFR	TGADGLLHARFYAWFEEQLRE	20.3	18.4	21.1	0.9	1.1
H2CB-3-D3-IGFR	RRSDSSLHRSFYDWFSVQLLN	22.5	18.3	21.3	0.9	1.2
H2CB-4-F2-IGFR	SESKYLLHSGFYGWFEAQLRG	18.0	16.8	18.3	0.9	1.1

FIG. 1N-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXFHXXFYXWFXXXXXX	--	--	--	--	--
H2CB-4-H1-IGFR	HGVIRADHTGFYGWFSKQLSD	18.3	15.3	16.5	0.9	1.1
H2CB-4-F9-IGFR	LINA.VFRRGFYAWFEEQVSK	22.9	14.4	15.3	0.9	1.1
H2CB-4-E10-IGFR	LQRYIGFHDPFYDWFSRALSG	26.1	20.1	24.5	0.8	1.2
H2CB-4-F8-IGFR	MRTAELFHVGFYDWFDAQLMD	21.5	14.8	19.0	0.8	1.3
H2CB-3-A8-IGFR	WAPPDALHGQFYRWFQRQLDQ	20.7	14.7	18.2	0.8	1.2
H2CB-4-F1-IGFR	AVHAATFHDDFYRWFEQVVGS	22.2	14.6	18.8	0.8	1.3
H2CB-3-C6-IGFR	FDAVHGFDGGFYGWFKRELQR	15.7	7.8	10.2	0.8	1.3
H2CB-4-E11-IGFR	QAGGMEFHGAFYNWFLQQLSG	26.1	17.6	24.1	0.7	1.4
H2CB-3-D6-IGFR	GRSVSRMNAEFYQWFGHQLAA	21.6	13.0	18.8	0.7	1.5
H2CB-4-F3-IGFR	AAVNSLFHDEFYLWFQDQLDG	17.3	11.1	16.4	0.7	1.5
H2CB-3-A4-IGFR	QLGMDWFHADFYEWFLAQLPS	27.4	11.0	14.8	0.7	1.3
H2CB-3-B1-IGFR	RLAGSGIHEGFYGWFVDQLLA	20.0	11.0	15.2	0.7	1.4
H2CB-3-C5-IGFR	GREIGGVHDGFYDWFRQQSEQ	19.9	10.5	15.6	0.7	1.5
H2CB-4-F6-IGFR	VRSEQRFDSSFYQWFNDLLMS	18.6	10.1	14.6	0.7	1.4
H2CB-3-B8-IGFR	QSPYGFFHDGFYRWFLQQTGM	20.7	6.9	9.5	0.7	1.4
H2CB-3-C7-IGFR	FQCGAAFHVDFYRWFTCQEQF	16.2	1.8	2.5	0.7	1.4
H2CB-4-H7-IGFR	GAFGSEFHEQFYRWFEDALSF	21.8	14.1	22.7	0.6	1.6
H2CB-4-F5-IGFR	EHTSYQIHRQFYEWFDRALGR	12.9	4.0	7.2	0.6	1.8
H2CB-4-G1-IGFR	SGTAADLHSRFYGWFALQARE	20.4	10.3	19.7	0.5	1.9
H2CB-3-D11-IGFR	EGFGVLFHGQFYRWFQLQLDG	24.1	8.8	18.6	0.5	2.1
H2CB-3-D7-IGFR	QQSAGHPHSSFYLWFSELLGA	22.1	6.5	13.6	0.5	2.1
H2CB-3-C10-IGFR	YLQRAGFHRSFYGWFDQALRD	21.7	5.1	10.4	0.5	2.0
H2CB-4-E3-IGFR	MWLWATLHSDFYSWFEQVVSG	20.3	4.6	8.9	0.5	1.9
H2CB-3-C1-IGFR	GANALGFKDRFYEWFAAQLWD	22.3	6.7	15.7	0.4	2.3
H2CB-4-G2-IGFR	GSGLYVFHWGFYDWFEQQMGG	19.9	3.3	10.7	0.3	3.3
H2CB-3-A11-IGFR	LDKGWGFDLQFYRWFEAATRA	23.9	2.5	7.7	0.3	3.1
H2CB-4-G5-IGFR	QRSAVEFHADFYDWFLRLLTP	19.3	2.5	7.9	0.3	3.1
H2CB-4-F12-IGFR	DQRMGSFHGEFYRWFEETLLS	16.7	1.7	5.4	0.3	3.1

FIG. 1N-3

Clone Design	Sequence X_n-FyxWF-X_n	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
20E2A-3-B11-IR	GRFYGWFQDAIDQLMPWGFDP	24.6	1.4	23.6	0.1	16.8
20E2Bβ-3-E3-IR	IQGWEPFYGWFDDVVAQMFEE	23.0	0.9	15.3	0.1	16.3
rB6-3-F6-IR	RYGRWGLAQQFYDWFDR	40.9	1.0	13.3	0.1	13.3
rB6-4-F9-IR	RGRLGSLSTQFYNWFAE	34.1	1.0	12.6	0.1	12.6
20E2Bα-3-A8-IR	ASAYTPFYQWFADVVSEYMQQ	35.4	7.4	34.4	0.2	4.6
A6L-4-F6-IR	PYRMEGTEKWNFYDWFVAQLQ	28.9	4.1	18.1	0.2	4.4
20E2Bα-4-H9-IR	SAVHFQFYKWFDNLLPVPLSA	37.8	9.4	26.7	0.4	2.9
20E2Bα-3-B1-IR	VPVNKSFYRWFQLVLGGSDDW	41.8	12.9	36.8	0.4	2.9
20E2Bβ-4-F9-IR	QSPRASFYGWFDDVLRAAGVV	25.9	4.2	10.1	0.4	2.4
20E2Bβ-3-E9-IR	TGFYEWFYEQLHSRMLPNPLD	27.0	7.7	17.2	0.5	2.2
20E2Bβ-3-E10-IR	RRGVGGFYGWFSQQLQGMGVA	22.2	2.6	5.5	0.5	2.1
20E2Bα-3-C12-IR	SSQDRRFYRWFEQAIVGGRDG	39.0	6.7	12.0	0.6	1.8
20E2Bβ-3-C12-IR	TRGQLGFYNWFQQALSTSGMG	20.2	2.2	3.8	0.6	1.8
20E2Bβ-3-E7-IR	CADLNAFYQWFCGVLDRGSDH	9.2	1.2	1.9	0.6	1.6
20E2Bβ-3-E11-IR	TLIQDQFYWWFSDLLSAEPGD	20.7	1.3	2.1	0.6	1.6
20E2Bα-3-B11-IR	IDQLDAFYRWFDGVMLGMGDP	36.0	20.7	32.8	0.6	1.6
NNKH-4-G2-IR	RGGGTFYEWFESALRKHGAG	10.8	6.3	8.9	0.7	1.4
20E2Bα-3-A7-IR	RGLDQDFYRWFQNLVGVEYDR	19.0	4.2	5.5	0.8	1.3
20E2Bα-4-G12-IR	MQGHRGFYGWFARVLEQDRGW	37.0	22.3	29.5	0.8	1.3
20E2Bα-3-C11-IR	ERLHLRFYEWFDTVIGQDGSD	37.3	26.8	34.8	0.8	1.3
20E2Bα-3-C10-IR	MHVQSDFYHWFQSLLGQGGPD	37.7	24.8	30.5	0.8	1.2

FIG. 10-1

Clone Design	Sequence X_n-FyxWF-X_m	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
20E2Bα-3-D7-IR	TMGTQGFYRWFQNVVKEHLSG	35.4	26.9	31.3	0.9	1.2
20E2Bα-3-A12-IR	ITHNRGFYSWFLDVVQGGAGA	31.7	22.0	23.3	0.9	1.1
20E2Bα-3-D10-IR	VRRDAGFYQWFADILTQLDFE	32.7	27.3	29.1	0.9	1.1
20E2Bα-4-G7-IR	MQLQDEFYNWFRGIMLNDGQD	34.2	29.0	30.7	0.9	1.1
20E2Bα-4-F5-IR	GIRSSGFYQWFDRVLAGVGDG	33.8	32.1	34.0	0.9	1.1
20E2Bα-3-C9-IR	ANLNSQFYSWFASVTGEASPS	39.4	33.2	35.5	0.9	1.1
20E2Bα-3-A4-IR	QSPRASFYGWFDDVLRAAGVV	38.2	31.6	35.9	0.9	1.1
20E2Bα-4-E12-IR	MQRNQGFYSWFDDLVSSTVGV	36.0	30.8	29.7	1.0	1.0
20E2Bα-4-E11-IR	ASGFDPFYAWFLEQLRVANGS	35.1	31.2	30.7	1.0	1.0
20E2Bα-4-E8-IR	SGTPYGFYRWFQSALASATSG	36.1	30.5	30.7	1.0	1.0
20E2Bα-4-H10-IR	QGVEGGFYEWFDRAMGDVRPW	38.9	30.6	30.7	1.0	1.0
20E2Bα-4-F6-IR	DNMSGGFYRWFAQVVADSGGD	34.9	33.2	32.0	1.0	1.0
20E2Bα-4-G4-IR	RGTDDTFYGWFDQLLQGWCDD	34.1	33.7	32.2	1.0	1.0
20E2Bα-4-F8-IR	TVDHTQFYDWFSRVLGESGSA	37.7	32.0	32.7	1.0	1.0
20E2Bα-4-G5-IR	GRQDREFYYWFELQAGGMDGD	34.9	33.9	33.4	1.0	1.0
20E2Bα-3-B10-IR	RLLLGGFYEWFDQVLKETKEV	38.2	34.9	33.6	1.0	1.0
20E2Bα-3-C7-IR	GVLSTGFYEWFALQLHGLAAG	37.6	34.2	34.8	1.0	1.0
20E2Bα-3-C5-IR	PAVGQSFYGWFEAVLRGSKAG	40.4	36.0	35.6	1.0	1.0
20E2Bα-3-B9-IR	SNGISGFYEWFAAQVQTSDFQ	39.6	35.8	37.1	1.0	1.0
A6L-4-F11-IR	LLGLSQAAYANFYDWFVSQLA	33.1	4.6	4.6	1.0	1.0
20E2Bα-3-C2-IR	VPNSWMFYNWFAEQIEGSEGE	44.1	40.0	38.1	1.0	1.0
20E2Bα-3-B2-IR	ARRADGFYDWFREQVSGSAVQ	43.1	40.1	39.0	1.0	1.0
20E2Bα-4-G2-IR	GVVEGTFYEWFDRLLGGVQGD	34.1	33.6	29.8	1.1	0.9
20E2Bα-4-H6-IR	SHLTDPFYQWFVDQLRAGVRG	39.4	36.0	31.9	1.1	0.9

FIG. 10-2

Clone Design	Sequence X_n-FyxWF-X_n	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
20E2Bα-4-H5-IR	RSNDDAFYRWFSNILQVDGGG	38.7	35.1	32.3	1.1	0.9
20E2Bα-4-G3-IR	DSDGAQFYIWFEDQLRSAGWD	35.5	36.1	32.7	1.1	0.9
20E2Bα-4-H4-IR	PGLHRAFYQWFAEAVRSANKE	38.8	37.9	35.0	1.1	0.9
20E2Bα-3-C1-IR	SLGQGGFYDWFASQVGGADI	43.7	42.1	39.0	1.1	0.9
20E2Bα-4-E6-IR	CGQTQSFYQWFCEVMRVESGD	38.0	34.3	29.7	1.2	0.9
H5-3-D5-IR	IVVPGDTQGVNFYDWFVKQLQ	43.8	21.8	18.2	1.2	0.8
JBA5-3-D9-IR	RDVSMGSASTNFYDWFVQQLG	38.3	29.8	25.3	1.2	0.8
20E2Bβ-4-G6-IR	SQAGSAFYAWFDQVLRTVHSA	22.4	6.2	1.9	3.3	0.3
20E2Bβ-4-H10-IR	SNGISGFYEWFAAQVQTSDFQ	23.5	32.2	9.7	3.3	0.3
rB6-4-G8-IR	RRDRGGLDVFFYQWFMD	--	--	--	--	--

FIG. 10-3

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
R20β-4-F8-IR	XXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
	HLCVLEELFWGASLFGYCSG	39.1	1.8	27.7	0.1	15.4

FIG. 2A

Clone Design	Sequence HLCVLEELFWGASLFGYCSG	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
F815-4-H9-IR	PLCVLEELFWSTPLFGQCSY	34.9	0.9	37.6	<0.1	40.8
F815-3-B1-IR	HLCVLEELFWGASLFAQCVG	31.7	0.9	35.8	<0.1	39.3
F815-3-D1-IR	DLCVLEELFWGASRFGQCSG	30.4	0.9	33.5	<0.1	38.9
F815-3-D4-IR	HLCVLEELFWGASLFGQCAG	31.5	0.9	33.6	<0.1	38.8
F815-3-C5-IR	HLCVVEELFWGASLFGQCSG	31.1	0.8	31.2	<0.1	38.5
F815-4-H3-IR	NLCDLEVLFWGASLFRQCSG	33.7	1.0	37.2	<0.1	38.4
F815-3-A5-IR	PLCVLEEQFWGASLFGQCSG	37.4	1.1	40.9	<0.1	38.3
F815-3-D7-IR	QLCVLEELFWGASEFGQCSG	33.6	0.9	34.3	<0.1	38.3
F815-3-A1-IR	HLCELEELFWGASLFGQCSG	29.8	0.9	34.8	<0.1	38.0
F815-4-H4-IR	PLCVLEELFWGESLFGQCSG	31.1	0.9	32.7	<0.1	38.0
F815-3-A3-IR	HLCVLEELFWGASRFGQCSG	32.8	1.0	39.1	<0.1	37.9
F815-3-B3-IR	KLCVLEELFWGASLFGQCSG	33.7	1.0	37.5	<0.1	37.5
F815-3-A4-IR	YLCVLEELSWGASLFGQCSG	32.5	1.0	36.9	<0.1	37.5
F815-3-D2-IR	HLCVLEELLWGASLFAQCSG	31.9	0.9	34.1	<0.1	37.4
F815-3-C4-IR	QLCVLEQLFWGESLFGQCSG	31.6	0.8	31.8	<0.1	37.4
F815-3-B4-IR	HLCVLEELFWGGNLFSQCSG	33.8	1.0	36.7	<0.1	37.3
F815-3-C1-IR	HLCVLEELFWGASLYGQCSG	29.0	0.9	35.0	<0.1	37.3
F815-4-G9-IR	SLCALEEQFWGAALFGYCSG	36.5	1.0	38.9	<0.1	37.1
F815-4-G6-IR	HLCVLEEQFWGASLFDGCAG	34.9	1.0	36.4	<0.1	37.0
F815-3-A8-IR	QLCVLEELFWGASLFGQCSG	34.7	1.1	39.3	<0.1	36.9
F815-4-G5-IR	PLCVLEELFWGAALFGQCSG	26.5	1.0	35.1	<0.1	36.8
F815-3-B5-IR	HLCVLEELFWGASLFGQCTG	33.2	0.9	34.1	<0.1	36.8
F815-4-F4-IR	PLCVLEELFWGGSLFGQCSG	28.6	0.8	30.0	<0.1	36.7
F815-3-A2-IR	QLCVLEELVWGASLFGQCSG	32.5	1.0	36.6	<0.1	36.6
F815-3-B6-IR	HLCVVEELIWGASLFGQCSR	31.6	0.9	32.9	<0.1	36.5
F815-4-H7-IR	DLCVLEELFWGASLFGQCAG	33.7	1.0	37.6	<0.1	36.4
F815-4-H8-IR	QLCVLEERFWGASLFGQCSG	35.8	1.0	37.0	<0.1	36.4
F815-4-G7-IR	NLCVLEELFWGAALFGQCSG	33.7	1.0	35.8	<0.1	36.3

FIG. 2B-1

Clone Design	Sequence HLCVLEELFWGASLFGYCSG	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
F815-3-A6-IR	QLCVLEELFWGSSLFGQCSG	34.6	1.1	39.0	<0.1	36.2
F815-3-D3-IR	DLCVVEELFWGKSLFGQCSG	33.8	1.0	36.2	<0.1	36.2
F815-3-B12-IR	DLCVLEELFWGSSLFGQCSG	33.2	1.0	35.7	<0.1	36.2
F815-4-G10-IR	YLCVLEEQFWGASLFRQCFG	35.4	1.0	37.2	<0.1	36.1
F815-4-E3-IR	HLCVLEELLWGSSLFGQCSG	32.4	1.0	35.0	<0.1	36.1
F815-4-E6-IR	PLCGLEELFWGASLFGQCSD	33.2	1.0	34.5	<0.1	36.1
F815-4-F1-IR	HLCVLEELFWGSSLFAQCSG	29.4	0.9	32.5	<0.1	36.0
F815-4-G8-IR	PLCAIEELFWGAALFGQCSG	36.8	1.1	38.2	<0.1	35.9
F815-4-H12-IR	HLCVLEEQFWGASLFGDCSG	30.5	0.9	31.9	<0.1	35.9
F815-4-G3-IR	PLCVLEELFWGAPLFGQCSD	31.4	1.0	35.7	<0.1	35.7
F815-3-C2-IR	DLCGLEELFWGAALFGQCTS	32.3	1.0	36.1	<0.1	35.6
F815-4-E10-IR	QLCVLEKQLWGASLFWQCSG	35.4	1.0	36.5	<0.1	35.4
F815-3-A12-IR	HLCVLEELFWGASLYGQCPG	32.1	1.0	36.3	<0.1	35.3
F815-3-B8-IR	HLCVLEELFWGASLFDQCSG	33.6	1.0	35.8	<0.1	35.3
F815-3-B2-IR	HLCVLEELLWGASLFGQCSG	31.0	1.0	35.3	<0.1	35.3
F815-3-C3-IR	PLCVLEELFWGVSLFGQCGG	30.1	1.0	35.3	<0.1	35.3
F815-3-A7-IR	HLCVLEELFWGASQWGQCSG	33.1	1.0	35.8	<0.1	35.2
F815-4-F9-IR	RLCVLEEQFWGGALFGQCSG	33.4	1.0	35.7	<0.1	35.2
F815-3-B7-IR	QLCVLEELFWGVSLFAQCSG	32.0	1.0	33.5	<0.1	35.0
F815-4-E4-IR	HLCVLEELFWGAALFGQCFG	28.0	1.0	33.4	<0.1	35.0
F815-4-E12-IR	YLCVLEELFWGASQFGQCSG	28.0	0.9	30.2	<0.1	34.8
F815-4-F8-IR	HLCVLEELYWGASLFGQCSG	33.8	1.0	35.2	<0.1	34.7
F815-3-C7-IR	HLCVLEERFWGVSLFGQCSG	33.9	1.0	34.7	<0.1	34.7
F815-4-F10-IR	PLCVLEELFWGASRFGQCSG	32.7	1.0	34.2	<0.1	34.7
F815-3-D11-IR	HLCVLEDLFWGASLFDQCSG	35.4	1.1	37.3	<0.1	34.6
F815-4-E7-IR	HLCDLEVLFWGASLFGQCSG	30.3	0.9	32.2	<0.1	34.6
F815-3-A10-IR	QLCILEEQFWGTSLFGYCSG	34.0	1.1	36.4	<0.1	34.3
F815-3-B11-IR	ALCVLEELFWGESLFGQCSG	33.7	1.1	36.3	<0.1	34.2

FIG. 2B-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	HLCVLEELFWGASLFGYCSG	--	--	--	--	--
F815-4-F11-IR	RLCVLEERFWGAALFGQCSG	31.8	1.0	33.7	<0.1	34.2
F815-3-A9-IR	PLCVLEELFWGASLFGQCSG	31.9	1.0	35.5	<0.1	34.1
F815-4-G11-IR	SLCVLEELFWGGSRFGQCSG	32.3	1.0	34.4	<0.1	33.9
F815-3-D8-IR	HLCLLEEQFWGASLFGYCFE	32.3	1.0	33.3	<0.1	33.7
F815-4-G4-IR	HLCVLEEQFWGASLFGQCSG	23.8	1.0	32.2	<0.1	33.7
F815-3-C8-IR	DLCLLEELLWGASRFGQCSG	33.9	1.0	35.1	<0.1	33.6
F815-4-G12-IR	YLCVLEERFWGASLFGQCSG	31.7	1.0	33.5	<0.1	33.5
F815-3-D12-IR	HLCVLEEQFWGASLFGSCSG	33.3	1.0	34.8	<0.1	33.4
F815-4-F7-IR	QLCVLEEQLWGASLFGQCSG	33.3	1.0	34.3	<0.1	33.4
F815-4-F2-IR	HLCVLEELF*GESLFGYCSG	26.1	1.0	33.8	<0.1	33.3
F815-3-B9-IR	HLCVLEELFWGASLFGQCSG	33.6	1.1	35.7	<0.1	33.2
F815-4-H2-IR	PLCVLEELFWGASHFGQCSG	36.1	1.2	38.4	<0.1	33.0
F815-4-E11-IR	HLCVLEELVWGASLFGQCAG	33.2	1.1	35.4	<0.1	33.0
F815-4-G1-IR	QLCVLEELIWGASLFGQCAG	27.9	1.0	31.5	<0.1	32.8
F815-3-A11-IR	HLCGLEELFWGASLFGQCSG	37.7	1.2	40.1	<0.1	32.7
F815-4-F6-IR	HLCVLEELVWGESLFGQCSG	32.3	1.1	34.6	<0.1	32.6
F815-3-D9-IR	RLCVLEELYWGASLFGQCSG	31.4	1.0	32.5	<0.1	32.5
F815-3-C11-IR	RLCILEELFWGASLFGQCSG	33.4	1.1	35.7	<0.1	31.9
F815-4-G2-IR	HLCVLEELFWGATLFDQCSG	30.2	1.1	34.3	<0.1	31.4
F815-3-C9-IR	HLCFLEELFWGASMFGQCSG	29.7	1.0	31.4	<0.1	31.0
F815-4-H10-IR	HLCIVEELFWAAPLFGQCSG	31.9	0.9	27.6	<0.1	29.4
F815-4-F3-IR	HLCVLEELWWGASLFAQCSA	19.4	1.0	28.0	<0.1	28.9
F815-4-F5-IR	NLCALEELFWGASQFRYCPG	12.3	0.9	24.8	<0.1	26.8
F815-4-H1-IR	RLCVLEELFWGASLFGQCSG	6.9	1.0	15.8	0.1	16.5
F815-4-E5-IR	PLCVLEELFWGASLFGQCPG	3.5	1.0	13.6	0.1	14.0
F815-4-H5-IR	NLCVLEELFWGASLFGQCSG	5.5	1.0	13.1	0.1	13.5
F815-3-C10-IR	QLCVLG#RFWGGSLCGYCSD	3.5	1.1	5.2	0.2	4.5

FIG. 2B-3

Clone Parental/Design	Sequence HLCVLEELFWGASLFGYCSG	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		39.1	**1.8**	**27.7**	**0.1**	**15.4**
F815-4-F11-IGFR	PLCFLQELFGGASLGGYCSG	33.4	12.3	1.0	12.3	0.1
F815-4-E12-IGFR	FMCGLQELVGGAALLGHCSG	33.7	15.1	1.7	8.9	0.1
F815-4-H10-IGFR	PLCFLQELFGGGSLSGYCSG	30.1	8.5	1.0	8.5	0.1
F815-4-B7-IGFR	FLCGLEELAWGVSRSGYCFG	35.2	23.9	4.8	5.0	0.2
F815-3-B5-IGFR	PLCFLAELFSGSALGGDCSR	33.9	4.8	1.0	4.8	0.2
F815-4-D12-IGFR	PLCVLQELFGGGSLGGYCSG	33.6	7.0	1.8	3.9	0.3
F815-4-C11-IGFR	QLCVLE#LFWGACLFGYCAG	13.9	4.6	1.8	2.6	0.4
F815-4-C7-IGFR	FLCGLQELSGVASLFGQCSG	16.8	2.0	1.0	2.0	0.5
F815-4-E7-IGFR	RVCVLEQLVWGASLFGA*SG	26.9	3.8	1.9	2.0	0.5
F815-4-G7-IGFR	FYCGLEELSWGAALFGYCSG	30.4	9.0	5.0	1.8	0.6
F815-4-A10-IGFR	FLCGLEELSQGAVLFGHCYG	30.8	3.7	2.2	1.7	0.6
F815-3-B3-IGFR	HLCVLVGLFWDASLFGQCSG	7.6	1.0	2.0	0.5	2.0
F815-3-G1-IGFR	QRCIRAALFWCATLLGGCAG	20.5	1.0	2.0	0.5	2.0
F815-4-G12-IGFR	HQCIPDGMSQGAALRGNCSD	7.6	1.0	2.5	0.4	2.5
F815-3-H1-IGFR	HLCVLEDELWGVSLFGYCSS	18.4	1.0	6.8	0.1	6.8

FIG. 2C

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	**HLCVLEELFWGASLFGYCSG**	**39.1**	**1.8**	**27.7**	**0.1**	**15.4**
F820-4-B5-IR	HLCMLEEQFWGASLFSRCSG	28.1	0.9	17.9	<0.1	21.1
F820-4-A2-IR	TCAFWKNGSGVRRCSVTAVV	34.0	1.6	22.7	0.1	13.9
F820-4-E2-IR	PLCGLKN.SGVRLCSSPALV	21.3	0.7	9.0	0.1	13.4
F820-4-D10-IR	PLCLQEELFWGASLFGYCSG	34.1	1.0	12.1	0.1	12.1
F820-4-H7-IR	PLCDLEELFWGASLFGDCPG	14.2	0.6	6.5	0.1	11.6
F820-4-G6-IR	DLCVLEELFWDGSLFASCSG	14.0	0.5	6.1	0.1	11.5
F820-4-C2-IR	PLCVLEEQLWGTALFGSCTG	38.1	1.2	11.8	0.1	9.9
F820-4-B4-IR	PLCLVEELLWGASLFSQCTG	15.1	0.7	6.4	0.1	8.7
F820-4-C7-IR	PLCDLEELYWGAALFGSCSG	46.3	2.7	22.2	0.1	8.2
F820-4-F10-IR	GLCFLEEQFWGTSLFRDCPG	14.5	0.6	4.7	0.1	8.0
F820-4-G5-IR	PLCVVEELFWGASLYGQCSG	8.8	0.6	4.4	0.1	7.5
F820-4-F2-IR	RLCVLEELFWGASRFRGCSG	11.7	0.6	4.2	0.1	7.4
F820-4-H8-IR	PLCVLEELHWGAALFGYCSG	16.0	0.6	4.7	0.1	7.3
F820-4-D7-IR	NLCVVEELFWGASLFPNCSG	14.5	0.8	5.9	0.1	7.1
F820-4-B2-IR	QLCVLEELFWGASMFEDCSG	5.0	0.4	2.4	0.2	6.9
F820-4-C3-IR	HLCVLEEQFWGASLFGQCSG	37.5	1.1	7.5	0.2	6.6
F820-4-H4-IR	PLCVLEEIYWGAALFGDCYG	21.2	1.1	6.4	0.2	5.9
F820-4-B10-IR	PLCVLEELFWGLSLDKNCS	7.5	0.7	3.7	0.2	5.6
F820-4-A5-IR	QLCVLEELFWGASLFSGCSG	5.3	0.8	4.4	0.2	5.2
F820-4-F6-IR	PLCDLEALFWGESLFGGCSG	5.7	0.6	3.0	0.2	4.9
F820-4-F1-IR	HLCVLEEMFWGTSHFDGCSG	9.1	1.0	4.7	0.2	4.7
F820-4-A3-IR	DLCVLEELFWGAPLFGLCSG	5.9	0.8	3.5	0.2	4.5
F820-4-D1-IR	DLCVLEELFWGVALYGGCSG	25.7	2.3	10.5	0.2	4.5
F820-4-F5-IR	QLCVLEELYWGASLFGHCSG	3.7	0.6	2.7	0.2	4.2
F820-4-F12-IR	HLCVLEDRFWGASLFGPCSG	11.3	0.6	2.2	0.3	3.5
F820-4-A11-IR	HLCGMEEMFWGVALFRNCSG	7.6	0.8	2.7	0.3	3.5
F820-4-E8-IR	PLCVLEQLYWGESLFVYCSG	8.0	1.2	4.3	0.3	3.5
F820-4-H3-IR	HLCLLEELFWGEALWGYCSG	17.5	2.6	9.0	0.3	3.4

FIG. 2D-1

Clone Parental/Design	Sequence HLCVLEELFWGASLFGYCSG	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
F820-4-A8-IR	QLCVMEELFWGASRFGQCSG	6.4	0.7	2.4	0.3	3.4
F820-4-G1-IR	HLCVLEELFWGASMFGQCSG	3.9	0.6	1.9	0.3	3.4
F820-4-F3-IR	QLCVLEEMFWGGSRFVQCSA	9.8	1.3	3.6	0.4	2.9
F820-4-D6-IR	PLCILEELFWGEALFDQCGA	5.4	1.2	3.2	0.4	2.6
F820-4-A1-IR	YLCVQEELFWGASLFGYCSV	25.5	2.4	6.1	0.4	2.5
F820-4-H2-IR	HLCALEEAFFGPSLFNSCQG	15.9	1.6	4.1	0.4	2.5
F820-4-F4-IR	HLCVLEERFWGASLFGQCSG	6.8	1.9	4.7	0.4	2.5
F820-4-B6-IR	QLCDLEELFWGASLFGYCPG	4.1	0.8	1.9	0.4	2.4
F820-4-B11-IR	HLCVLEERFWGASIWGSCSG	22.2	3.1	7.0	0.4	2.3
F820-4-H6-IR	QLCVLEELFWGGSLWGQCSR	4.1	1.1	2.4	0.5	2.2
F820-4-H9-IR	PLCVLEELFWGAAQFGQCSG	3.1	0.9	1.9	0.5	2.1
F820-4-D3-IR	QLCDLEERFWGVSLFGLCSG	4.6	1.3	2.5	0.5	1.9
F820-4-C1-IR	QLCVLEEVFWGASLFGLCTG	13.0	1.1	2.1	0.5	1.9
F820-4-D12-IR	QL.DLNTWSGLCLCSVTVRV	10.4	1.2	2.0	0.6	1.7
F820-4-B8-IR	DLCVLEESLWGKALFGYCSD	7.2	2.2	3.4	0.6	1.5
F820-4-C6-IR	HLCVLEEVFWGSSMFGDCSG	13.9	2.5	2.8	0.9	1.1
F820-4-C10-IR	HLCDLEELFWGASLFGDCQG	5.3	2.6	2.9	0.9	1.1
F820-4-D4-IR	QLCVLDALMWGGCRLGHQCG	3.5	2.3	2.1	1.1	0.9
F820-4-E1-IR	QLCVLEEKFWGTSLFGDCMG	1.6	1.6	1.5	1.1	0.9
F820-4-B3-IR	HLCVLEEVFWGAAQFGSCSG	15.9	0.6	5.0	1.2	0.8
F820-4-D2-IR	QLCVLEELFWGPSMFGYCSG	7.8	3.2	2.5	1.3	0.8
F820-4-C5-IR	HLCDLEELFWGASGFAQCYG	21.5	4.0	2.3	1.8	0.6

FIG. 2D-2

Clone Design	Sequence	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
	HLCVLEELFWGASLFGYCSG	--	--	--	--	--
A6L-3-C4-IR	DLCVLEERFWGASLFGQCSG	36.9	1.0	40.5	<0.1	42.5
A6L-3-D7-IR	QLCVLEELHWGASLFGYCSG	38.6	1.0	40.1	<0.1	40.7
A6L-3-A1-IR	PLCVLEEQFWGASLFGQCSG	39.6	1.1	44.8	<0.1	40.6
A6L-3-C1-IR	YLCDLEERFWGASLFGQCSS	37.3	1.0	40.3	<0.1	40.3
A6L-3-D5-IR	HLCLLEERFWGSSQFGFCSG	42.9	1.1	44.4	<0.1	40.2
A6L-3-A4-IR	HLCVLEELFWGASQFGQCSG	26.7	1.1	42.2	<0.1	40.2
A6L-3-D3-IR	HLCYLEERFWGASLFGQCSG	34.6	0.9	36.9	<0.1	39.8
A6L-3-B1-IR	HLCVMEELFWGTSLFGQCTG	33.9	1.0	38.7	<0.1	39.3
A6L-3-B5-IR	HLCVLEERFWGASLFGQCSG	35.3	1.1	42.4	<0.1	38.6
A6L-3-B2-IR	HLCVLEERFWGASLFSQCSG	38.1	1.1	42.7	<0.1	37.7
B6H-4-G12-IR	HLCVLEELFWGASLFGQCSG	31.6	1.1	39.6	<0.1	36.7
B6C-4-H10-IR	QLCLLEELFWGAASFGQCSG	38.5	1.1	41.1	<0.1	36.5
B6H-4-G8-IR	HLCVLEEMFWGASLFGQCSG	31.7	1.1	39.7	<0.1	36.2
A6L-3-D6-IR	HLCDLEELFWGASLFSQCSR	35.5	1.0	37.2	<0.1	36.1
B6C-4-F1-IR	QLCVLEELFWGASQFGYCSG	32.9	1.1	38.7	<0.1	35.8
B6C-4-H3-IR	QLCALEEQFWGASLFSQCSG	37.4	1.2	40.5	<0.1	34.8
B6H-4-E8-IR	QLCVLEELFWGASLFGYCSG	30.2	1.0	35.7	<0.1	34.3
B6C-4-G1-IR	HLCVLEEWFWGDSLFGQCSR	34.9	1.2	40.2	<0.1	33.7
B6H-4-E9-IR	HLCVLEERFWGASLFGQCSG	34.4	1.2	38.8	<0.1	33.2
B6C-4-F5-IR	QLCELEEVFWGASLFDYCSG	34.7	1.2	39.6	<0.1	32.8
B6C-4-F11-IR	HLCVLEELFWGASRFGQCSG	34.0	1.2	37.2	<0.1	31.7
B6C-4-E6-IR	HLCVLEELFWGASLFGQCSA	32.3	1.2	37.4	<0.1	30.6
B6C-4-E12-IR	HLCVLEELIWGASRFGQCSG	30.9	1.1	33.3	<0.1	30.2
B6C-4-G10-IR	HLCVLEELFWGGSLFIQCSG	33.0	1.3	40.3	<0.1	30.1
B6C-4-F8-IR	QLCVLEEQFWGASLFGNCSG	36.4	1.4	39.8	<0.1	29.3
20C-3-B5-IR	HLCVLEERFWGAALFGQCSG	26.6	1.1	32.5	<0.1	29.2
B6C-4-G3-IR	HLCILEEMFWGASLFGQCGG	34.0	1.4	38.8	<0.1	28.3
20C-3-B7-IR	PLCVLEELVWGASLFVQCSG	29.5	1.2	32.9	<0.1	28.3

FIG. 2E-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	HLCVLEELFWGASLFGYCSG	--	--	--	--	--
20C-3-B4-IR	NLCVLEELFWGESLFGQCSG	28.9	1.1	31.1	<0.1	28.0
20C-3-C11-IR	HLCVLEEQFWGGSLFGYCSR	30.2	1.1	31.0	<0.1	27.7
B6C-4-G2-IR	HLCFLEEVFWGAALFAQCSG	29.4	1.3	35.3	<0.1	27.5
20C-3-B8-IR	HLCDLEVLFWGSALFGQCSG	28.5	1.1	31.2	<0.1	27.4
20C-3-C10-IR	HLCVMEELFWGASLFGQCSG	32.1	1.2	33.6	<0.1	27.1
20C-3-B6-IR	HLCVLEERFWGASLFWQCSG	29.7	1.2	31.9	<0.1	26.7
A6L-3-A3-IR	HLCVLEEQYWGESLFGYCSG	14.4	1.1	28.3	<0.1	26.5
A6L-3-B3-IR	PLCVLEEQFWGASLFAYCSS	38.7	1.7	43.4	<0.1	26.3
20C-3-A5-IR	QLCVLEELFWGESLFAQCLG	22.9	1.1	27.6	<0.1	26.0
20C-3-B11-IR	HLCVLEELFWGQSLFGHCSD	30.0	1.3	32.7	<0.1	25.8
20C-3-B3-IR	HLCVLEELVWGASLFGFCSG	29.3	1.2	31.2	<0.1	25.7
20C-3-C12-IR	LLCVLEEQFWGASLFGQCSG	29.6	1.3	31.8	<0.1	24.8
20C-3-C3-IR	RLCVLEELFWGESLFGQCSG	30.1	1.2	30.1	<0.1	24.3
20C-3-C2-IR	HLCVLEEMFWGASLFGNCSG	29.9	1.3	29.8	<0.1	23.8
20C-3-A11-IR	ELCFLEELFWGASLFGQCSG	25.9	1.2	27.4	<0.1	23.0
20C-3-A4-IR	HLCVLEELFWGASLYGQCSS	27.2	1.2	27.5	<0.1	22.9
20C-3-A6-IR	HLCVLEELFWGASLFAQCPG	26.1	1.2	27.5	<0.1	22.8
B6C-4-E4-IR	NLCVLEELFWGASEFGQCSG	34.5	1.7	39.1	<0.1	22.7
20C-3-A9-IR	DLCVLEEQLWGASLFRYCSG	29.7	1.3	29.3	<0.1	22.7
B6C-3-C5-IR	HLCVLEEQFWGVALFGNCSG	33.5	1.7	37.7	<0.1	22.5
20C-3-B1-IR	HLCVLEVQIWGASLFGQCSG	30.2	1.2	26.7	<0.1	22.0
20C-3-A10-IR	HLCVLEERFWGGALFGQCTA	29.0	1.3	28.5	<0.1	21.5
20C-4-F1-IR	HLCDLEELFWGASLFGQCSG	29.1	1.4	29.5	<0.1	20.7
20C-4-E1-IR	QLCVLEELFWGTSLFAGCSG	28.3	1.4	29.7	<0.1	20.6
20C-3-B12-IR	QLCGLEELFWGASLFGYCSA	27.0	1.3	25.8	<0.1	20.2
20C-3-A8-IR	HLCVLEELFWGASLFGQCSS	21.1	1.1	21.2	0.1	20.0
20C-3-A7-IR	FLCVLEELYWGASQFGQCSG	21.9	1.3	23.0	0.1	18.3
B6C-4-E10-IR	HLCVLEEQFWGASLFGYCSG	35.2	2.2	38.0	0.1	17.5

FIG. 2E-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	HLCVLEELFWGASLFGYCSG	--	--	--	--	--
20C-3-A1-IR	RLCALEELFWGASLFGQCSG	21.0	1.1	17.6	0.1	16.6
20C-3-C1-IR	HLCVLEELFWGAALFHQCSG	30.6	1.4	21.9	0.1	16.1
A6L-3-D2-IR	RLCVLEEQFWGASLFGQCSG	7.0	1.1	14.9	0.1	14.1
B6C-4-G12-IR	QLCVLEELFWGSSRLGYCSG	31.1	2.5	33.5	0.1	13.6
B6H-4-F9-IR	DLCVLEELFWGASLFGQCSG	39.3	3.6	43.1	0.1	12.1
B6C-4-E3-IR	QLCLLEEQFWGGSLFGQCSG	34.6	5.3	40.0	0.1	7.6
20C-3-B10-IR	HLCVLEELFWGTSLFGQCSG	29.9	16.9	31.7	0.5	1.9
20C-3-A3-IR	RLCVLEELVWGASLFDQCSR	28.4	19.1	25.3	0.8	1.3

FIG. 2E-3

Clone Design	Sequence XXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
R20α-3-20A4-IR	EIEAEWGRVRCLVYGRCVGG	50.2	1.6	23.1	0.1	14.4
R20β-4-A7-IR	EIEAEWGRVRCLVYGRCVGG	44.2	1.3	24.0	0.1	18.5
R20β-4-D8-IR	WLDQEWAWVQCEVYGRGCPS	44.8	1.4	24.2	0.1	17.3

FIG. 3A

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	**WLDQEWAWVQCEVYGRGCPS**	**44.8**	**1.4**	**24.2**	**<0.1**	**17.3**
D815-4-A8-IR	WLDLEWAQVQCEVYGRGCPS	48.0	1.0	48.4	<0.1	48.4
D815-4-D10-IR	WLDQEWAQVQCEVFGRGCPS	49.2	1.0	48.2	<0.1	48.2
D815-4-D9-IR	WLDQEWQQVQCQVYGRGCTS	47.5	1.0	48.0	<0.1	48.0
D815-4-A11-IR	RLDEEWARVQCEVWGRGCRS	47.9	1.0	48.0	<0.1	48.0
D815-4-E12-IR	WLEQEWAWIQCEVYGRGCPS	49.0	1.0	47.6	<0.1	47.6
D815-4-B7-IR	WLEQEWAQVQCEVYGRGCPS	45.4	1.0	47.2	<0.1	47.2
D815-4-D11-IR	WLDEEWEWIQCKVYGRGCPA	49.5	1.0	47.0	<0.1	47.0
D815-4-D12-IR	WLEQEWAWVQCEVYGRGCQS	48.1	1.0	46.6	<0.1	46.6
D815-4-F8-IR	WLDQEWAWIQCEVYGRGCPA	47.8	1.0	46.4	<0.1	46.4
D815-4-A9-IR	SLDWEWAWLQCEVYGRGCPS	47.7	1.0	45.8	<0.1	45.8
D815-4-E9-IR	WLEQEWEQVRCLVYGRGCPP	47.8	1.0	45.8	<0.1	45.8
D815-4-B10-IR	WLDQEWAWVQCEVYGRGCPY	49.0	1.0	45.6	<0.1	45.6
D815-4-H8-IR	WLDQEWAGVLCEVYGRGCPS	49.0	1.0	45.6	<0.1	45.6
D815-4-E10-IR	SLDKEWEWVLCVVYGRGCPS	47.0	1.0	45.6	<0.1	45.6
D815-4-D7-IR	WLEQEWAQVQCEVYGRGCRS	44.5	1.0	45.4	<0.1	45.4
D815-4-G9-IR	WLEEEWAQVQCAVYGRGCSS	44.2	1.0	44.2	<0.1	44.2
D815-4-G12-IR	WLDQEWALVQCEVYGRGCPS	44.3	1.0	43.7	<0.1	43.7
D815-4-E11-IR	WLDQEWAWVQCEVYGRGCPS	45.5	1.0	43.0	<0.1	43.0
D815-4-H7-IR	WLEQEWAWVQCEVYGRGCAS	46.2	1.0	43.0	<0.1	43.0
D815-4-F12-IR	WLDQEWAWVECEVYGRRCPS	47.2	1.0	42.6	<0.1	42.6
D815-4-E8-IR	WLDQEWAWVECQVYGRGCPS	47.9	1.0	42.6	<0.1	42.6
D815-4-F9-IR	QLDQEWAWVLCKVYGRGCPS	46.4	1.0	41.8	<0.1	41.8
D815-4-A10-IR	WLDHE*AWVQCEVYGRGCPS	47.3	1.0	41.2	<0.1	41.2
D815-4-C7-IR	QLEQEWAWVRCEVYGRGCSS	37.7	1.0	40.0	<0.1	40.0
D815-4-H10-IR	WLDQEWAWVQCQVYGRGCLS	47.0	1.0	39.8	<0.1	39.8
D815-4-C9-IR	WLDQEWAWVRCEVYGLGCPS	44.2	1.0	39.8	<0.1	39.8
D815-4-F11-IR	WLDQEWAVMKCELYGRGCPS	40.4	1.0	39.2	<0.1	39.2
D815-4-H12-IR	WLEQEWAWVQCEVYGRGCLS	45.4	1.0	38.6	<0.1	38.6
D815-4-A7-IR	SLDQEWAWVQCEVYGRGCLS	37.3	1.0	37.3	<0.1	37.3
D815-4-H11-IR	WLDHEWAWVQCEVYGRGCTS	2.4	1.0	37.2	<0.1	37.2
D815-4-F7-IR	WLDVEWAWVQCEVYGRGCPS	32.4	1.0	34.7	<0.1	34.7

FIG. 3B-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	**WLDQEWAWVQCEVYGRGCPS**	--	--	--	--	--
D815-4-G8-IR	QLDQEWARVRCEVWGRGCSS	27.8	1.0	33.6	<0.1	33.6
D815-4-G7-IR	WLDLEWAQVQCKVYGRGCPS	34.7	1.0	32.3	<0.1	32.3
D815-4-G11-IR	WLDEEWAWVQCQVYGRGCPS	30.7	1.0	28.6	<0.1	28.6
D815-4-E7-IR	WLDQEWAWVQCEVWGRGCAF	33.0	1.0	26.4	<0.1	26.4
D815-4-A12-IR	WLDREWAQVQCEVYGRGCLS	28.4	1.0	19.0	0.1	19.0
D815-4-B11-IR	WLDAEWEWVQCEVYGRGCRP	22.1	1.0	18.8	0.1	18.8
D815-4-D8-IR	SLDREWAYVQCQVYGRGCSS	20.8	1.0	14.6	0.1	14.6

FIG. 3B-2

Clone Parental/Design	Sequence WLDQEWAWVQCEVYGRGCPS	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		44.8	1.4	24.2	0.1	17.2
D820-3-H2-IR	RLDLEWANIQCEVYGRGCPS	23.9	1.0	40.0	<0.1	40.0
D820-3-C4-IR	WLEQEWARVQCEVYGRGCSS	31.0	1.0	39.5	<0.1	39.5
D820-3-C3-IR	WLEQEWILVECEVYGRGCPT	35.2	1.0	39.4	<0.1	39.4
D820-3-G6-IR	WLEQEWAQVQCEVWGRGCPS	33.8	1.0	38.8	<0.1	38.8
D820-3-D2-IR	WLDQEWEWIQCEVYGRGCPL	35.6	1.0	37.8	<0.1	37.8
D820-3-D3-IR	LLDEEWAQIECEIYGRGCPS	34.8	1.0	37.7	<0.1	37.7
D820-3-B5-IR	ALEEEWAWVQCEVYGRGCHF	34.1	1.0	37.1	<0.1	37.1
D820-3-E2-IR	C?EQEWGLVQCEVYGRGCPS	34.4	1.0	37.0	<0.1	37.0
D820-3-B3-IR	WLEQEWAYVQCEVYGRGCPS	33.6	1.0	36.7	<0.1	36.7
D820-3-B6-IR	WLEHEWAQVQCEVWGRGCPY	31.2	1.0	36.6	<0.1	36.6
D820-3-D4-IR	WLEQEWAEVRCEVYGRGCPR	32.0	1.0	36.2	<0.1	36.2
D820-3-C2-IR	?LEQEWAWVQCEVYGRGCPS	33.7	1.0	35.6	<0.1	35.6
D820-3-F6-IR	WLEQEWAGIQCKVYGRGCPS	30.8	1.0	35.2	<0.1	35.2
D820-3-D5-IR	RLEQEWAQVQCEVWGRGCLP	30.5	1.0	34.8	<0.1	34.8
D820-3-F5-IR	QLDHEWAGIQCEVWGRGCPS	29.8	1.0	34.6	<0.1	34.6
D820-3-H3-IR	WLEQEWAQIQCEVYGAGCRS	30.2	1.0	33.8	<0.1	33.8
D820-3-G2-IR	SLEQEWAWVQCVVYGRGCPI	31.3	1.0	33.0	<0.1	33.0
D820-3-H6-IR	WLEQEWDQVLCEVYGRGCPY	30.3	1.0	32.2	<0.1	32.2
D820-3-F3-IR	WLEQEWAQV?CEVYGRGCA?	28.6	1.0	30.7	<0.1	30.7
D820-3-B4-IR	WMDQEWAWVQCEVYGRGCPS	33.1	1.0	30.5	<0.1	30.5
D820-3-C5-IR	QLDQEWAWIQCEVYGRNCRT	29.1	1.0	30.3	<0.1	30.3
D820-3-F4-IR	TLEQEWAQVICEVYGRGCLS	25.9	1.0	29.5	<0.1	29.5
D820-3-H5-IR	RLEQEWAQVQCEVWGRGCLS	26.3	1.0	28.6	<0.1	28.6
D820-3-A6-IR	WLDQEWALVQCEVYGRGCPA	24.8	1.0	26.0	<0.1	26.0
D820-3-A2-IR	WLDQEWAQIQCHVWGRGCPA	23.7	1.0	25.6	<0.1	25.6
D820-3-G5-IR	WLEQEWAWVQCEVYGRGCPS	22.6	1.0	25.0	<0.1	25.0
D820-3-G3-IR	RLEEEWAWVQCQVYGRGCPS	22.2	1.0	23.9	<0.1	23.9
D820-3-E3-IR	WLEQEWVRIQCEVYGRGCPS	20.6	1.0	22.7	<0.1	22.7

FIG. 3C-1

Clone	Sequence	Ratios over Background			Comparisons	
Parental/Design	WLDQEWAWVQCEVYGRGCPS	E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
D820-3-E5-IR	WLEQEWTWVQCEVYGCGCPS	44.8	1.4	24.2	0.1	17.2
D820-3-D1-IR	WLEKEWAGVQCEIYGRGCPS	25.9	1.0	22.6	<0.1	22.6
D820-3-E1-IR	WLEEEWAWVRCEVYGRGCQS	27.3	1.0	22.4	<0.1	22.4
D820-3-F1-IR	WLEHEWAQIQCELYGRGCTY	22.4	1.0	21.9	<0.1	21.9
D820-3-B2-IR	ALEEEWAWVQCEVYGRGCPS	22.0	1.0	21.0	<0.1	21.0
D820-3-A3-IR	WLEQEWAQVQCEVYGRGCPS	13.1	1.0	18.4	0.1	18.4
D820-3-H4-IR	WLDDEWAQIQCEIYGRGCQS	23.5	1.0	18.4	0.1	18.4
D820-3-G1-IR	QLEEEWAGVQCEVYGRECPS	25.6	1.0	17.5	0.1	17.5
D820-3-C1-IR	WLEQEWLLVQCGVYGRGCPS	14.5	1.0	16.3	0.1	16.3
D820-3-A1-IR	WLDQEWAWIQCEVYGRGCRS	27.8	1.0	13.9	0.1	13.9
D820-3-A5-IR	WLEQEWAQVQCEVSGRGCPS	14.7	1.0	12.8	0.1	12.8
D820-3-H1-IR	W?DQEWALIQCEVYGRGCPS	6.4	1.0	6.3	0.2	6.3
D820-3-A4-IR	SLDEEWAGVLCEVYGRGCPF	13.7	1.0	6.2	0.2	6.2
D820-4-E12-IR	SVDQELEWLMCHFQGRVCPS	6.0	1.0	4.3	0.2	4.3
D820-4-B12-IR	WLEQERAWIWCEIQGSGCRA	34.9	9.0	10.9	0.8	1.2
		32.2	8.6	1.0	8.6	0.1

FIG. 3C-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	**WLDQEWAWVQCEVYGRGCPS**	**44.8**	**1.4**	**24.2**	**0.1**	**17.3**
D820-3-D5-IGFR	WVNQALGGVQSDVQGRRCQS	29.6	3.8	1.0	3.8	0.3
D820-3-E4-IGFR	LLDHEWPWVGCEVCGRGSLS	27.1	3.2	1.0	3.2	0.3
D820-3-C5-IGFR	WLHQELAWVRGEGYPRGRRS	25.0	3.1	1.0	3.1	0.3
D820-3-F4-IGFR	WLGHDWAWIQCEVYGLGCPC	3.9	2.7	1.0	2.7	0.4
D820-3-F6-IGFR	WIDQEGVRVQCEA*GRAFPS	26.7	2.6	1.0	2.6	0.4
D820-3-G4-IGFR	WRDEEWAWVQGVVQGRGWPA	3.8	2.6	1.0	2.6	0.4
D820-3-E2-IGFR	RLGVEWSWFQRKVYGRDSTS	15.3	2.6	1.0	2.6	0.4
D820-3-G6-IGFR	WLAQGWAGVQCVVYGRGCRN	20.3	2.4	1.0	2.4	0.4
D820-4-E11-IGFR	WLEEE*AGIQCQV?GRGCPS	12.6	1.0	3.0	0.3	3.0
D820-4-H11-IGFR	WLDQEWEWVQCEVWGRGCLS	8.1	1.0	4.6	0.2	4.6
D820-4-D11-IGFR	RLEQEWALIQCEVYGRGCPS	4.5	1.0	5.3	0.2	5.3
D820-4-A8-IGFR	WLEEEWAQVQCQVYGRGCAS	3.2	1.0	5.5	0.2	5.5
D820-4-F9-IGFR	WLDLE*EWLQCEVYGRGCAT	9.4	1.0	5.8	0.2	5.8
D820-4-C8-IGFR	WLEQEWVQVRCEVYGRGCPS	11.6	1.0	5.9	0.2	5.9
D820-4-D9-IGFR	WLEEEWAQVQCEVYGRGCPS	10.1	1.0	8.9	0.1	8.9
D820-4-D7-IGFR	WLDQEWARVQCEVWGRGCTY	34.1	3.5	33.4	0.1	9.5
D820-4-H9-IGFR	YLD?EWAWVQCEVYGLGCQS	18.4	1.0	10.1	0.1	10.1
D820-4-E10-IGFR	WLDVE*AWVQCEVWGRGCPS	26.7	2.6	27.0	0.1	10.4
D820-4-E7-IGFR	WLEQEWER?QCEVYGRGCPP	31.9	3.0	32.2	0.1	10.7
D820-4-H8-IGFR	WLEEEWAQVQCEVYGRGCLS	16.1	1.0	11.7	0.1	11.7
D820-4-A11-IGFR	WLDQEWAWIQCEVYGRGCPS	8.0	1.0	12.5	0.1	12.5
D820-4-C9-IGFR	?LEHEWAQIQCEV?GRGCQS	19.6	1.0	14.9	0.1	14.9
D820-4-E9-IGFR	WL?QEWAWIQCEVYGRGCPF	19.3	1.0	17.3	0.1	17.3
D820-4-B10-IGFR	WLD?EWAWVQCEVYGRGCPS	19.3	1.0	21.5	<0.1	21.5
D820-4-F10-IGFR	GLEQGCPWVGLEVQCRGCPS	27.8	1.0	25.7	<0.1	25.7
D820-4-B9-IGFR	WLEEEWAWVQCEVYGHGCPS	31.7	1.0	26.5	<0.1	26.5
D820-4-G8-IGFR	WLDQEWAQIQCEVYGRGCSS	25.6	1.0	29.3	<0.1	29.3

FIG. 3D-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	**WLDQEWAWVQCEVYGRGCPS**	**44.8**	**1.4**	**24.2**	**0.1**	**17.3**
D820-4-G9-IGFR	WLDQEWAQVQCEVWGRGCPS	36.8	1.0	29.6	<0.1	29.6
D820-4-C10-IGFR	WLDLEWEFVQCEVYGRGCPT	32.6	1.0	31.3	<0.1	31.3
D820-4-A9-IGFR	WLEQEWASVQCEVYGRGCPS	20.4	1.0	31.4	<0.1	31.4
D820-4-B8-IGFR	WLDLEWEQIKCKVYGRGCPF	31.1	1.0	32.7	<0.1	32.7
D820-4-F8-IGFR	WLEQEWAQIQCQIYGRGCPS	28.3	1.0	32.9	<0.1	32.9
D820-4-H7-IGFR	WLEQEWALVLCEVYGHGCPA	34.1	1.0	32.9	<0.1	32.9
D820-4-E8-IGFR	WLEQEWAQIQCEVWGRGCSS	26.6	1.0	33.2	<0.1	33.2
D820-4-G10-IGFR	WLE?EWEWVQCEVYGRGC?S	37.5	1.0	33.2	<0.1	33.2
D820-4-D10-IGFR	WLEQEWAQVQCDVYGRGCPS	36.6	1.0	33.5	<0.1	33.5
D820-4-D8-IGFR	WLEQE*ARVQCEVWGRGCPS	23.7	1.0	34.6	<0.1	34.6
D820-4-A10-IGFR	WL?QEWARVHCEVWGRP?QC	29.4	1.0	35.5	<0.1	35.5
D820-4-B7-IGFR	PLEHEWAWVQCVVYGRGCRS	35.4	1.0	36.9	<0.1	36.9
D820-4-E12-IGFR	SLE?EWAWVQCEV?GRGCP?	37.0	1.0	37.0	<0.1	37.0
D820-4-H10-IGFR	WLDQEWVRVQCEVWGRGCPS	36.8	1.0	37.1	<0.1	37.1
D820-4-F12-IGFR	SLDKEWAWVKCEVYGRGCPS	36.9	1.0	37.3	<0.1	37.3
D820-4-F7-IGFR	LGDQEWAWVEWEV#GRGWPS	34.4	1.0	37.5	<0.1	37.5
D820-4-G12-IGFR	WLEEEWAQIRCGVYGRGCPS	30.3	1.0	37.8	<0.1	37.8
D820-4-D12-IGFR	WLEEE*GWVQCEVWGRGCPP	37.2	1.0	38.6	<0.1	38.6
D820-4-A12-IGFR	CLDQEWA?VQCPVYGRGCPS	30.4	1.0	39.3	<0.1	39.3
D820-4-C12-IGFR	QLELEWARVQCEVWDRGCPS	37.1	1.0	39.6	<0.1	39.6
D820-4-A7-IGFR	RLEQEWAWIQCEVYGRGCRF	35.4	1.0	40.8	<0.1	40.8
D820-4-B12-IGFR	SLEHE*AWVQCKVYGRGC?S	36.2	1.0	41.4	<0.1	41.4

FIG. 3D-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	**WLDQEWAWVQCEVYGRGCPS**	**44.8**	**1.4**	**24.2**	**<0.1**	**17.3**
B6-4-G12-IR	WLDQEWAWIQCEVYGRGCPP	4.4	1.0	6.9	0.1	7.1
B6-3-A11-IR	WLDQEWAQVRCEVYGRGCPS	7.3	1.0	6.3	0.2	6.3

FIG. 3E

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
R20-4-C10-IGFR	PKGTRFRGDVDVWDGYSWLA	37.8	3.8	--	--	--

FIG. 4A-1

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Design	XXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
20F-4-B7-IGFR	TPIPAGGINIASWGGYTWLS	10.9	3.7	0.5	7.3	0.1
20F-4-E4-IGFR	HRGTVTGVWVARWPGYEWLS	8.9	4.7	0.7	6.3	0.2
20F-4-E12-IGFR	SDVWAQPQRRNDWPGYHWLS	9.7	4.7	0.8	6.0	0.2
20F-4-F4-IGFR	HRGTVTGVWVARWPGYEWLS	13.9	10.1	1.8	5.6	0.2
20F-4-F7-IGFR	SDVWAQPQRRNDWPGYHWLS	13.7	3.9	0.8	5.1	0.2
20F-4-E7-IGFR	RPHRINPQDDAVWPGYLWLG	7.2	2.5	0.5	4.7	0.2
20F-4-F11-IGFR	HRGTVTGVWVARWPGYEWLS	17.6	16.2	3.5	4.6	0.2
20F-4-D10-IGFR	FGRGYGGDGGGYWSGYEWLA	9.8	2.4	0.6	4.1	0.2
20F-4-B3-IGFR	DGLVVKSGREWPGYGWLER.A	17.3	14.4	3.6	4.0	0.2
20F-4-B12-IGFR	DGSIV.VSSSVGWPGYEWLM	10.1	9.9	2.4	4.0	0.2
20F-3-A9-IGFR	WQQANLSNGGGRWGGYDWLM	6.6	2.7	0.7	4.0	0.2
20F-4-G2-IGFR	FGRGYGGDGGGYWSGYEWLA	5.1	1.3	0.5	2.7	0.4
20F-4-D11-IGFR	VNYEMDRVPPMPWGGYWWLS	5.0	1.0	0.5	2.3	0.4
20F-4-G4-IGFR	MGGGLWVGVHIWPGYSWLSQ	3.9	0.9	0.5	1.8	0.6
20F-4-G12-IGFR	SDVWAQPQRRNDWPGYHWLS	3.2	0.9	0.6	1.5	0.7

FIG. 4A-2

Clone Design	Sequence XXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
R20β-4-A4-IR	WPGYLFFEEALQDWRGSTED	11.9	17.5	1.4	12.5	0.1
R20β-4-F2-IR	SMFVAGSDRWPGYGVLADWL	16.4	13.9	3.1	4.5	0.2
R20β-4-E8-IR	VRGFQGGTVWPGYEWLRNAA	41.0	34.9	3.6	9.7	0.1

FIG. 4B-1

Clone Design	Sequence XXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
20F-4-H10-IR	LDLASGDSWLGYDVLRGWLS	10.2	3.1	2.4	1.3	0.8
20F-4-C10-IR	IHSSDGIGAWGGYAWFRDVA	23.4	9.6	4.1	2.3	0.4

FIG. 4B-2

Clone Design	Sequence XXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
R20β-4-D10-IR	LGPLLRWGSEVCGVWPDLCE	21.5	1.0	8.0	0.1	8.0
R20β-4-D9b-IR	PFGFGGRWWGIPRMWWYRNS	32.6	6.8	15.1	0.5	2.2
R20β-4-H4-IR	WWWGGRNRWWLERWGLGGER	11.6	1.7	3.6	0.5	2.1
R20β-4-A2-IR	GRVALWGPVWPRWWFMSRPV	17.1	2.6	5.2	0.5	2.0

FIG. 4C

Clone Design	Sequence XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
R40-3-40A2-IR	RGTRTDRLWKSGGFAIVPRWPCFSYHCLVEWITKTGSPG	44.6	1.5	2.7	0.6	1.8
R40-4-40F10-IR	GRTSMAFVPPRHLQPELAPRPVRNHAWLVGGG	46.4	1.9	2.1	0.9	1.1

FIG. 4D

Clone Design	Sequence XXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag --	IGFsR --	IR --	Comparisons IGFR/IR --	IR/IGFR --
R20-4-F10-IGFR	CLGAGSFRAGILCLGGLPVS	35.5	6.0	--	--	--
R20-4-F7-IGFR	GFWATACGGLQICEELGLKP	29.1	4.7	--	--	--
R20-4-H9-IGFR	DLFCAYMAQALGLGQDLSCG	25.7	3.0	--	--	--
R20-3-A4-IGFR	RHLLLPQIWIAS*GGWGMG	15.6	2.7	--	--	--

FIG. 4E

Clone Design	Sequence XXXXCXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
20C-3-H3-IGFR	DHRLCGTDEYLMQDLFVRGLCRLIW	28.5	26.6	1.0	26.6	<0.1
20C-3-F4-IGFR	GLLFCKQLFTLAGLQPEAGCVSSSR	34.4	27.5	1.2	23.1	<0.1
20C-4-C10-IGFR	IWIACLDELLRGQVWSSCRRRAPIG	35.5	24.4	1.3	19.2	0.1
20C-3-G5-IGFR	DWLRCLGVILSGGLTELANTGCVQG	29.3	21.1	1.1	18.7	0.1
20C-3-A2-IGFR	WFSFCLGGLLQAQEWSVWGRDVGCI	33.9	18.3	1.1	16.9	0.1
20C-3-B4-IGFR	GYSWLRDVLMEKQAQLKREGSVGRQ	39.8	29.1	1.9	15.2	0.1
20C-3-C6-IGFR	FLTRLLERLGLS*ERGEAGGPYAQA	34.8	20.9	1.4	14.9	0.1
20C-3-E2-IGFR	FSGFCMGLERLSQVSLGYCGAGQGG	34.8	28.1	2.0	14.2	0.1
20C-3-A3-IGFR	ISFRCQLFVLAGMHPCPVDVGGEGF	33.7	14.3	1.2	12.4	0.1
20C-3-B1-IGFR	NTPNCSQDWGQESGFMALLLALTCK	30.2	9.8	0.9	11.2	0.1
20C-3-F5-IGFR	LQGFCELLATVTGVTGLGCLDYQPI	35.5	31.9	3.9	8.2	0.1
20C-4-A7-IGFR	GSSICNLLARAQIVELALCEMGVQE	33.3	19.3	2.8	6.9	0.1
20C-4-F8-IGFR	LSFACLLSQLSGVVLPDCLLGED	30.5	27.7	5.3	5.2	0.2
20C-4-G11-IGFR	GEHFCQLLMSLCGDDCGPVNCGGGS	24.7	13.3	2.8	4.7	0.2
20C-3-E1-IGFR	GWFECLLASLVLQVPQGRSRASAVC	34.0	5.1	1.6	3.1	0.3
20C-3-B6-IGFR	YRQECACSVGAVGFLCGLACLARSG	37.3	32.8	13.7	2.4	0.4

FIG. 4F-1

Clone Design	Sequence XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	Ratios over Background E-Tag	IGFsR	IR	Comparisons IGFR/IR	IR/IGFR
		--	--	--	--	--
40F-4-D1-IGFR	LSCLAYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE	4.9	4.6	0.3	13.1	0.1
40F-4-B1-IGFR	GLDHSDAVGVHLGFAWPA.ARGRWEAGGLEDTWAGYDWL	4.1	3.0	0.2	13.1	0.1
40F-4-D10-IGFR	W.GYAWLS	4.9	4.5	0.4	11.7	0.1
40F-3-A3-IGFR	LSCLAYSRHGIWRPSTDLGLGRSVGEGSVSTRWRGYDWFE	2.6	2.0	0.3	7.9	0.1
40F-4-C4-IGFR	EAMAVGLQCPARFVRAAAHGDGGSWGQDHV.AWGGYWWLG	3.8	2.0	0.5	4.1	0.2

FIG. 4F-2

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	**HLCVLEELFWGASLFGYCSG**	**39.1**	**1.8**	**27.7**	**0.1**	**15.4**
F815-4-G11-IGFR	HFYVLVERLSGASLFGSGSA	34.6	7.9	1.0	7.9	0.1
F815-3-D1-IGFR	HRFVREGLLWGAYQFCYCSG	14.9	1.0	2.0	0.5	2.0
F815-4-C12-IGFR	FQSLLEELVWGAPLFRYGTG	35.2	1.0	2.0	0.5	2.0
F815-4-A11-IGFR	HLSVLEELSWGASLFGQWAG	5.4	1.0	2.1	0.5	2.1

FIG. 4G

Clone	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
Parental/Design	**HLSVLEELSWGASLFGQWAG**	**5.4**	**1.0**	**2.1**	**0.5**	**2.1**
NNKH-4-A9-IR	NLCRLEELAWGASLFGQCAG	16.3	1.0	2.7	0.4	2.6
NNKH-4-H4-IR	APVSTEELRWGALLFGQWAG	15.6	1.0	2.6	0.4	2.5
NNKH-4-B3-IR	HLSVLEERWWRESLFGQWAG	13.6	2.8	6.7	0.4	2.3
NNKH-4-E1-IR	HLSVLEERWWRAALFGQWAG	13.9	4.8	9.5	0.5	2.0
NNKH-4-E7-IR	HLSILEEQWWRESLFGQWAG	16.9	1.3	2.3	0.6	1.8
NNKH-4-G3-IR	HMSVEELSWWASLFGKQAG	11.3	1.3	2.3	0.6	1.7
NNKH-4-B6-IR	HLSELEERWWRATLFGQWAG	13.2	1.3	2.1	0.6	1.7
NNKH-4-A10-IR	HLSVLEELWWRESLFGQWAG	15.4	2.0	3.2	0.6	1.6
NNKH-4-A5-IR	HLSLLEEQWWRESLFGQWAG	14.6	4.6	6.9	0.7	1.5
NNKH-4-F11-IR	HLSVLEERWWRETLFGQWAG	14.0	3.1	3.9	0.8	1.3
NNKH-4-C9-IR	HLSVLEEQWWRESLFGQWAG	14.3	2.3	2.9	0.8	1.3
NNKH-4-D12-IR	HLSVLEEQWW.ESLFGQWAG	12.0	1.4	1.7	0.8	1.2
NNKH-4-D10-IR	HLSVLEELWWREALFGQWAG	13.6	1.2	1.5	0.8	1.2
NNKH-4-E5-IR	HLSVLEERWWRATLFGEWAG	14.5	1.4	1.6	0.9	1.1
NNKH-2-A6-IR	HL.VLEELLWGVSLFRQWAG	8.4	1.4	1.5	1.0	1.1
NNKH-4-F6-IR	HLSALEEQWWRATLFGQWAG	14.1	2.8	2.9	1.0	1.0
NNKH-4-C7-IR	HLSVLEERWWRATLLESGQ	14.7	1.4	1.4	1.0	1.0
NNKH-4-F7-IR	HLSALEELWWRETLFGQWAG	14.1	7.5	7.0	1.1	0.9
NNKH-4-F8-IR	HLSVLEELWWRESLFGKWAG	13.6	11.0	8.6	1.3	0.8
NNKH-4-E9-IR	HLSVLEEAWWRESLFGHWAG	15.5	7.9	6.0	1.3	0.8
NNKH-4-E6-IR	HMSEQEELWWRATLFGQWAG	18.2	3.8	2.7	1.4	0.7
NNKH-4-B7-IR	HLSVLEERWWRETLFGEWAG	16.5	12.9	8.2	1.7	0.6
NNKH-2-B3-IR	HRSVLKQLSWGASLFGQWAG	11.5	5.3	0.7	7.4	0.1

FIG. 4H

Clone Parental/Design	Sequence HLSVLEELSWGASLFGQWAG	Ratios over Background E-Tag	 IGFsR	 IR	Comparisons IGFR/IR	 IR/IGFR
NNKH-2-C5-IGFR	HL*VLEELSWGASLVGQWAV	5.4	1.0	2.1	0.5	2.1
NNKH-2-D9-IGFR	HLSVLEEL*LGASMFGLWAG	7.3	0.9	0.7	1.3	0.8
NNKH-2-H12-IGFR	HLSVLKELSW*ASLFGQWAG	4.1	0.5	0.4	1.3	0.8
NNKH-2-D10-IGFR	HLSALEELSWGASLFGQWAG	5.0	1.3	1.1	1.2	0.8
NNKH-2-G9-IGFR	HLSVLAELS*GALLFGQWAG	4.8	2.1	1.9	1.1	0.9
NNKH-2-C6-IGFR	RLSVLEQLSWGASLFGPWAG	1.9	1.4	1.3	1.1	0.9
NNKH-2-C7-IGFR	HL*VLVQPSWGASLFGQWAG	18.2	1.0	0.9	1.1	0.9
NNKH-2-F11-IGFR	HQSVLEELSR*ASLFGQWAG	21.8	1.3	1.3	1.0	1.0
NNKH-2-H3-IGFR	DMSVLGGLSWGA*LFGQWSG	6.7	1.3	1.4	0.9	1.1
NNKH-2-B8-IGFR	HLSVREGQLWRASMFGRWAG	4.7	0.7	0.8	0.9	1.1
NNKH-2-B12-IGFR	QLSVLVEL*WGASLFGPWAA	17.5	3.7	5.2	0.7	1.4
NNKH-2-F9-IGFR	HLSVGEELSW*VALLGQWAR	1.2	1.0	2.9	0.3	2.9
		3.7	0.6	2.1	0.3	3.5

FIG. 4I

D Name	Clonal Name	Formula #	$K_4(\mu M)$ HIR	PO_4	Fat Cell Assay	Activity	$K_4(\mu M)$ HIGFR	Ratio IGF/IR	Sequence
D101	20D3	1	0.51 0.27				13 11	25 41	KIGGQGQHQDGNFYDWFVEALAKK (ε-biotin)
D102	20D1	1	1.2 0.97				7.4 16	6.2 16	KVLQARHGCDSVSDCFYEWFAKK (ε-biotin)
D103	B8	1	0.74				15	20	KWSALLSVMDTGFYAWFDDAVKK (ε-biotin)
D104	E7	1	20				>20	>1	KGHSWALVRHVDRLFYEWFDLKK (ε-biotin)
D105	H8	1	2.8				12	4.3	KRDKPTDQEEQNWSFYEWFRHKK (ε-biotin)
D106	20F1	1	0.97				6.2	6.4	KVFWNCRSQQLDFYEWWFEQAAKK (ε-biotin)
D107	40G11	1	1.1	YES		Antagonist	9.7	8.8	KLESHYVVPQAALDRLFYSWFSKK (ε-biotin)
D108	3G11	1	2.3			Antagonist	19	8.3	KFYGWFSRQLSLTPRDDWGLPKK (ε-biotin)
D109	20H1	1	3.6			Antagonist	12	3.3	KSAPGLVSNKQDGLFYSWFREKK (ε-biotin)
D110	G3	1	0.84			Antagonist	1.4	1.7	KRGGGTFYEWFESALRKHGAGKK (ε-biotin)
D111	D2	1	0.62				3.2	5.2	KDPERMQSDVGFYEWFRAAVGKK (ε-biotin)
D112	IGFR C1 A65-4-C1	1	0.49 0.19			Neutral	0.05• 0.02•	0.1 0.1	DYKDCWARPCGDAANFYDWFVQQASKK (ε-biotin)
D113	IGFR H2 A65-4-1+2	1	0.75		-20 μ M	Agonist	5.4	7.2	DYKDVTFTSAVFHENFYDWFVRQVSKK (ε-biotin)
D114	IGFR A6	1	8.1			Neutral	>20	>2.5	SAKNFYDWFVKK (ε-biotin)
D115	IGFR D5	1	8.1				>20	>2.5	ADKNFYDWFMAAKK (ε-biotin)
D116	IGFR JBA5	9	4.4 cycli		>20 μ M	Agonist	8.1	1.8	DYKDLCQSWGVRIGWLAGLCPKK (ε-biotin)
D117	IGFR H2C	1	0.70	YES	-20 μ M	Agonist	6.1 5.1	8.6 8.5	FHENFYDWFVRQVSKK (ε-biotin)
D118	20E2	2	0.25	YES	-20 μ M	Antagonist	1.3	5.2	DYKDFYDAIDQLVRGSARAGGTRDKK (ε-biotin)
D119	20C11	2	0.25	YES	-20 μ M	Antagonist	13 2.5	2.9 0.8	KDRAFYNGLRDLVGAVYGAWDKK (ε-biotin)
D120	E8	10	0.37			Antagonist	2.2	5.9	KVRGFQGGTVWPGYEWLRNAAKK (ε-biotin)
D121	F2	10	1.1			Antagonist	7.4	6.7	KSMFVAGSDRWPGYGVLADWLKK (ε-biotin)
D122	20A4 (A7)	6	1.2 1.0			Antagonist	>20 >20	>17 >20	KEIEAEWGRVRCLVYGRCVGGKK (ε-biotin)
D123	D8	6	0.55 1.3			Antagonist	16 >20	29 >15	KWLDQEWAWVQCEVYGRGCPSKK (ε-biotin)
D124	F8	4	0.04• 0.09•				8.2 >20	200 >200	KHLCVLEELFWGASLFGYCSGKK (ε-biotin)
D125	IGFR E4	1	2.6				>20	>8	DYKDERSAAGFRGNFYDWFVAQVNKK (ε-biotin)
D126	IGFR D2C	1	1.4				18	13	LGENFYDWFVMQVRKK

FIG. 5A

Clonal Name	D or S name	Motif	Sequence	IR-Kd	IR-IC 50 Biacore	IR-IC 50 FP-S175	PO_4	Fat Cell Assay
20-E2	D118	B6	DYKDFYDAIDQLVRGSARAGGTRDK K-biotin	250 nM		2.8 nM	+	++
C1	D112	A6	DYKDCWARPCGDAANFYDWFVQQAS KK-biotin	490 nM			-	0
D8	D123	C-C LOOP	KWLDQEWAWVQCEVYGRGCPSKK	550 nM			0	-
E8	D120	GROUP 6	KRGFQGGTVWPGYEWLRNA	370 nM			-	-
F8	D124	C-C LOOP	KHLCVLEELFWGASLFGYCSGKK	40 nM			-	0
H2C	D117	A6	FHENFYDWFVRQQVSKK	700 nM	>5 μM	5 nM	++	++
KCF9			RLYYEWFWGQLEAQGRGGLS					
KC-C2		C-C-C	GLEQGCPWVGLEVQCRGCPS					
KCG7		B6	FYCGLEELSWGAALFGYCSG		>1 μM			
NG-C2		B6	GNGDGMFYQLLSLLVGRDMH					
NG-G33		A6	GHSQSCPESFYDWFAGQVSDPWWCW		2-4 μM	4.2 nM	+++	
NG-G8		B6	VEGRGLFYDLLRQLLARRQNG		>5 μM		-	
NG-G9		B6	RAMSFYDALVVLGLGPKK-Biotin				-	
RP-1		A6	GSRPVFHEQFYLWFVDQLGL		1 μM		+	
RP-2		A6	RSEASFHVEFYSWFEEQLRS		1 μM		+	
RP-3		A6	GRFYGWFQDAIDQLMPWGFD		>10 μM		-	
RP-4		B6	PPWGARFYDAIEQLVFDNL		5 μM		+	
RP-5		B6	AGVNAGFYRYFSTLLDWWDQGKK-Biotin		6 μM		-	
RP-6		B6+ C-C	TFYSCLASLLTGTPQPNRGPWERCRKK-Biotin				+++++	
RP-7		A6	AAVHEQFYDWFADQYKK					
RP-8		B6	QSFYDYIEELLGGEWKK		>5 μM		+	
RP-8#	S287	B6	QSFYDYIEELLGGEWEE					
RP-9		A6	GSLDESFYDWFERQLGKK			2.9 nM	++	
RP-10		B6	GSFYEALQRLVGGEQGKK		>10 μM		+	
RP-11		A6	QAPSNFYDWFVREWDKK		>10 μM		+	
RP-12		B6	DPFYQGLWEWLRESGKK					
RP-13		A6	ASGFPENFYDWFGRQLSLKK		>10 μM			
RP-14		A6	SACQFDCHENFYDWFARQKK		>10 μM			
RP-15		A6	SQAGSAFYAWFDQVLRTVKK					
RP-16		B6	VMDARDDPFYHKLSELVTKK					
RP-17		B6	QSDAFYSGLWALIGLSDGKK		>10 μM			
RP-18		B6	LQPCSGFYDYFWQRLHLGSKK					
RP-19		A6	LKDGFYDYFWQRLHLGSKK					
RP-20		B6	GSASFYDAIDRLLRMRIKK					
RP-24		GROUP 6	WPGYLFFEEALQDWRGSTED					
S167	S167	A6	AFYDWFAKK	>20 μM	No Binding			
S173	S173	RB6	LDALDRLMRYFEERPSL	1.2 μM				
S174	S174	RB6	PLAELWAYFEHSEQGRSSAH	16 μM				0
S175	S175	A6	GRVDWLQRNANFYDWFVAELG	230 nM	2-4 μM	0.9 nM	++	0
S176	S176	A6	NGVERAGIGDNFYDWFVAQLH	470 nM				+++

FIG. 5B

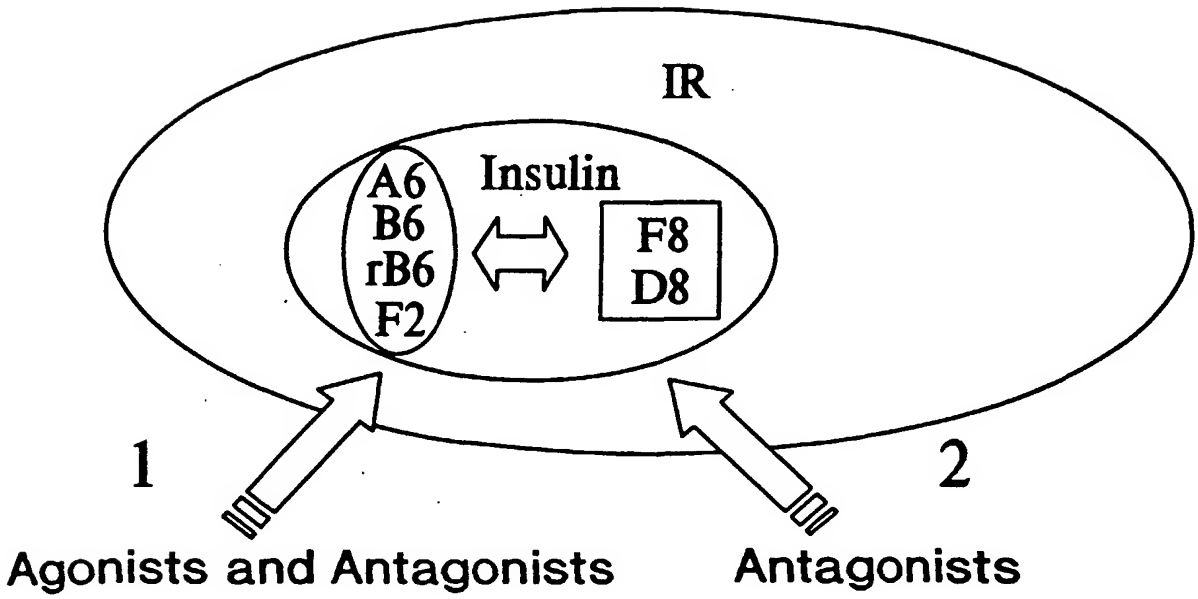

IR
Insulin
A6
B6
rB6
F2
F8
D8
1
2
Agonists and Antagonists
Antagonists

FIG. 6

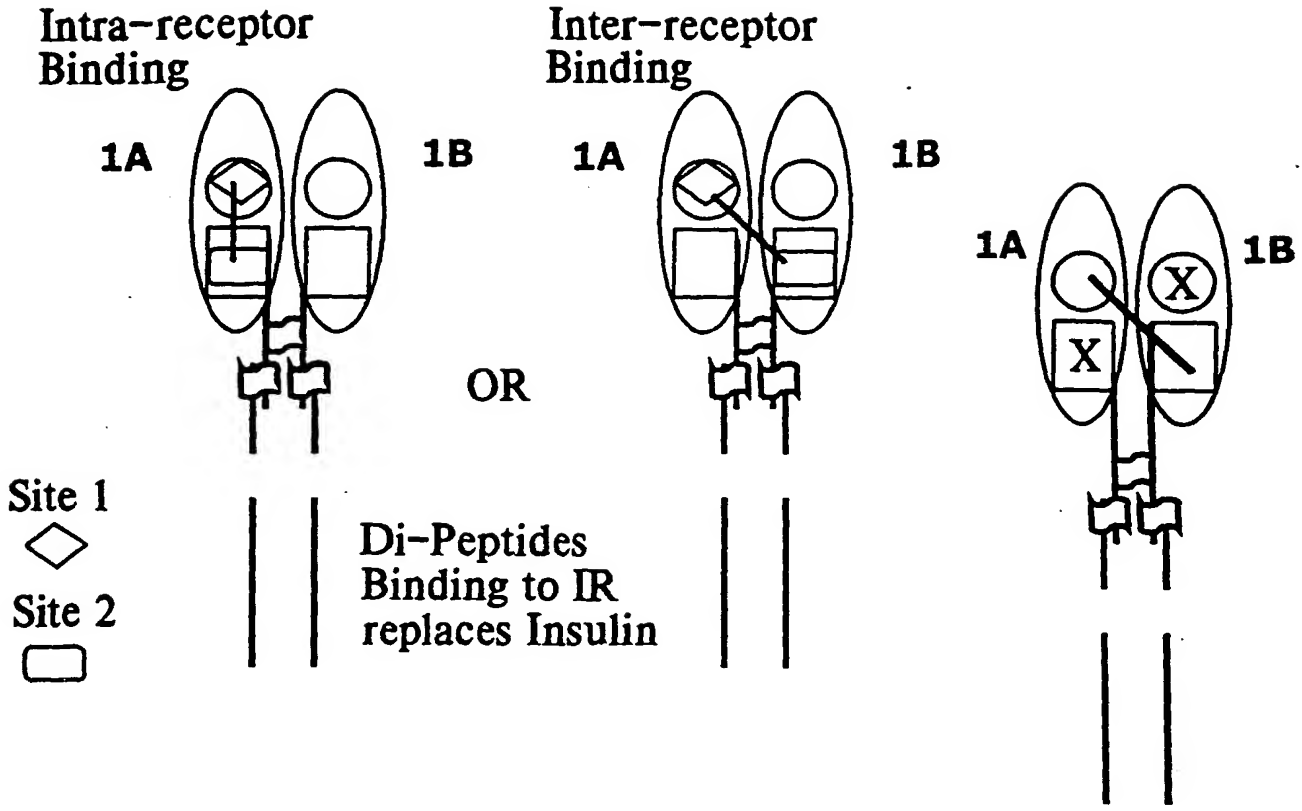

Intra-receptor Binding
1A
1B
OR
Inter-receptor Binding
1A
1B
1A
1B
X
X
Site 1
Site 2
Di-Peptides Binding to IR replaces Insulin

FIG. 7

Group 1 (Formula 1 Motif)		Target Found	IR	IGF
20D3*	IGGQGQHQDGNFYDWFVEALA	18	+	++++
20F1	VFWNCRSQQLDFYEWFEQAA	16	+	++++
G3	RGGGTFYEWFESALRKHGAG	8	+	+++
20H1	RVAGAISAPGLVSNKQDGLFYSWFRE	5	+	+++
20D1*	VLQARHGCDSVSDCFYEWFA	4	+	++++
D2	DPERMQSDVGFYEWFRAAVG	3	+	+++
B8	WSALLSVMDTGFYAWFDDAV	2	++	++++
C4	DIGSDGHGRRWDSFYRWFEM	2	+	+++
A8	IGGSFVEFYGWFNDQV	2	+	+++
E7	GHSWALVRHVDRLFYEWFDL	1	++	+++
C8	LPAGGAQGFAVRGFYEWFES	1	+	+++
H8	RDKPTDQEEQNWSFYEWFRH	1	+	+++
E2	SRDQTNFTFNSAGFYGWFER	1	+	+++
B12	GAFYRWFHEALVGSERVPDV	1	+	+++
D10-2	RIGGGWARSEGFYEWFVREL	1	+	++
G8	RMFYEWFWSQMGAGPTEGSA	1	+	++
H3	HEAFYDWFSALVDGGYELMG	1	+	++
3G11	FYGWFSRQLSLTPRDDWGLP	1	+	++
F4	GVGTLTMSSDAFYTWFV	1	+	++
E7-2	LGTSAGQGVGHRAFYQWFQS	1	+	+
40G11	<--ETLESHYVVPQ----------AALDRLFYSWFS	3	+	++++
40B2	IRDMHYVWVQDRDRYINGVRQWYISDRYNPGSAFYRWFID	2	+	++
40B12	RMGLQALAHYRKSA-----GPIFLSSGSVIKGSEGDPFYAWFRLQ	1	+	++

FIG. 8

Group 2: Formula 6 Motif

		Target		
		Found	IR	IGF
20A4*	EIEAEWGRVRCLVYGRCVGG	13	+++	0
D8	WLDQEWAWVQCEVYGRGCPS	3	+++	?

Group 3: Formula 2 Motif

		Target		
		Found	IR	IGF
20E2	*DYKD*FYDAIDQLVRGSARAGGTRD	1	+	++++
20C11	*DYKD*DRAFYNGLRDLVGAVYGAWD	1	+	++++
20A12	*DYKD*RLFYCGIQALGANLGYSGCV	1	+	++++
C6	*DYKD*FYSALWGLCGVTGCG	1	+	+++
A6	RGQSDAFYSGLWALIGLSDG	1	+	+++
40H4	RYFPFGGFYGNLDVLRWLRPYVASPRWGHWRPGGSLGKQPT	1	+	0

Group 5: Miscellaneous Motif 10

		Target		
		Found	IR	IGF
D9-2	PFGFGGRWWGIPRMWWYRNS	1	++	++
H4	WWWGGRNRWWLERWGLGGER	1	+	+

FIG. 9A

Group 4 and 6: Miscellaneous Motif 10

		Target		
		Found	IR	IGF
D10	LGPLLRWGSEVCGVWPDLCE	3	++	0
A2	GRVALWGPVWPRWWFMSRPV	1	++	+
F2	SMFVAGSDRWPGYGVLADWL	1	++	++
E8	VRGFQGGTVWPGYEWLRNAA	1	++	?
A4	WPGYLFFEEALQDWRGSTED	1	0	+++

Group 7: Formula 4 Motif

		Target		
		Found	IR	IGF
B6	ACSSFFVKGPEGFLQCLGSI	1	0	++
F8	HLCVLEELFWGASLFGYCSG	4	+++	+
40D6	PERGRGLRTAMQLMRRPRDWHFPHSLFWGAPPPLSG	1	0	0

FIG. 9B

FIG. 10A

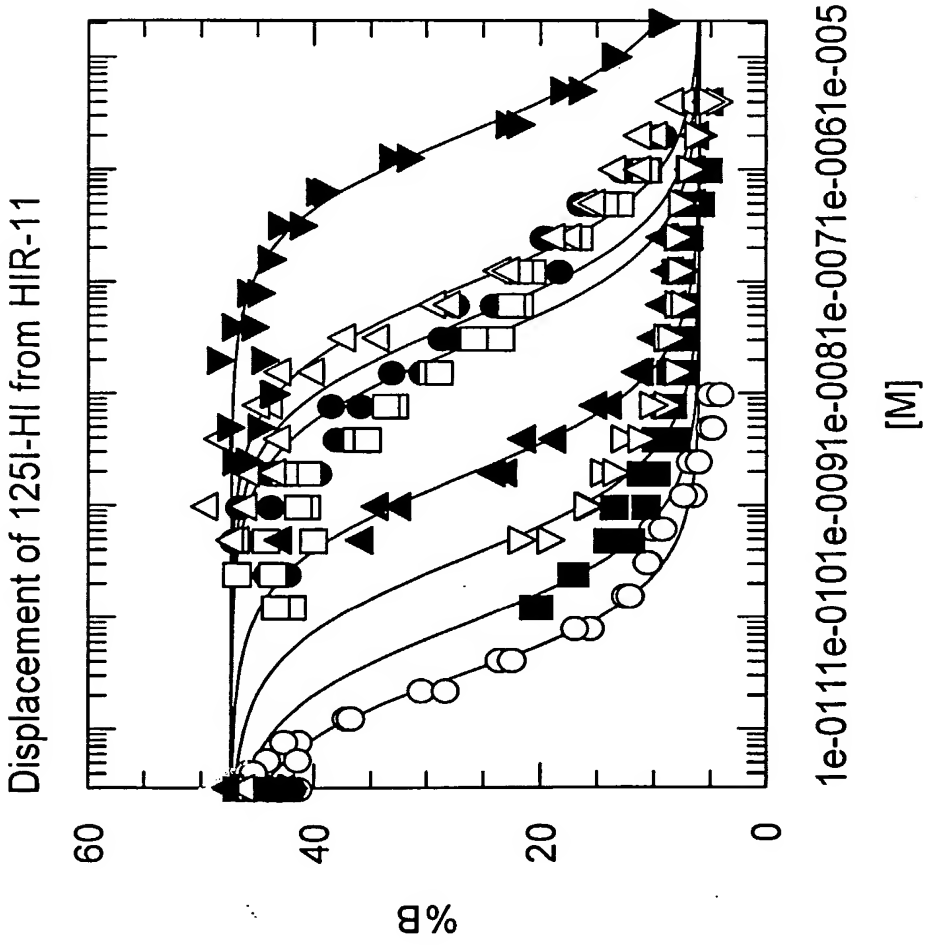


FIG. 10B

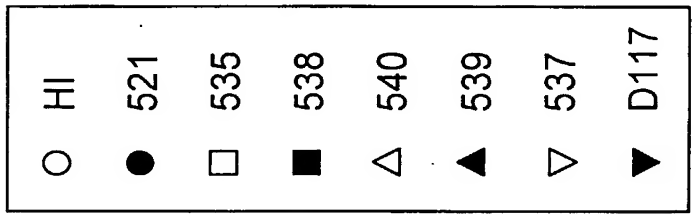


FIG. 10C

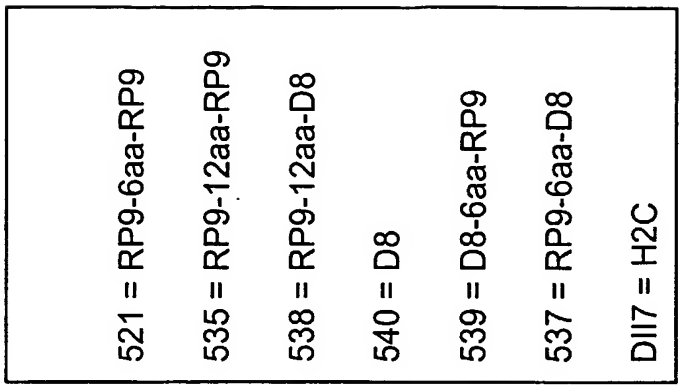


FIG. 11A

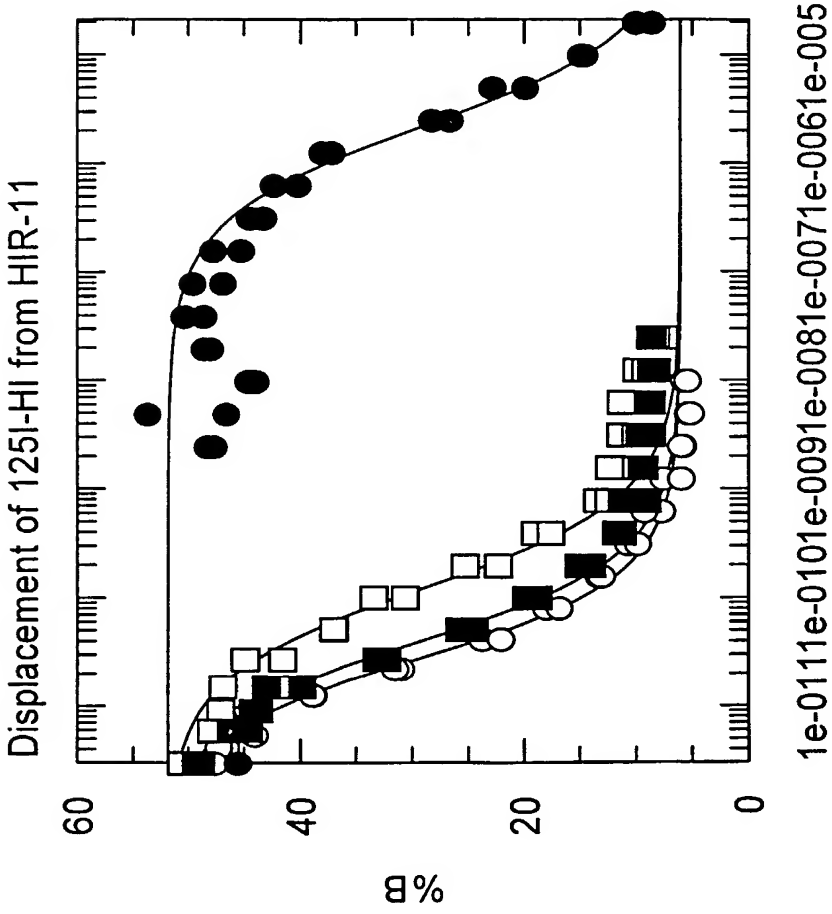


FIG. 11B

○	HI
●	D117
□	537
■	538

FIG. 11C

DII7 = H2C

537 = RP9-6aa-D8

538 = RP9-12aa-D8

Kd	
HI	1.1e-11
D117	1.1e-6
537	5.9e-11
538	1.7e-11

FIG. 11D

FIG. 12A

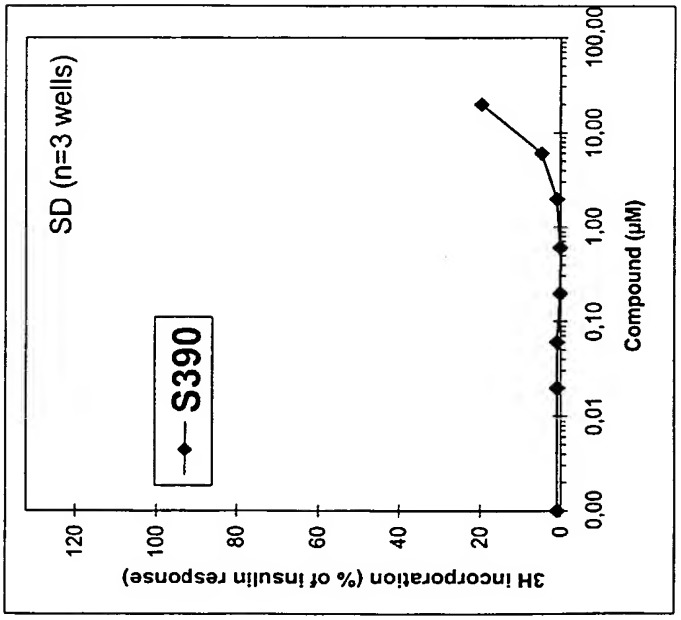
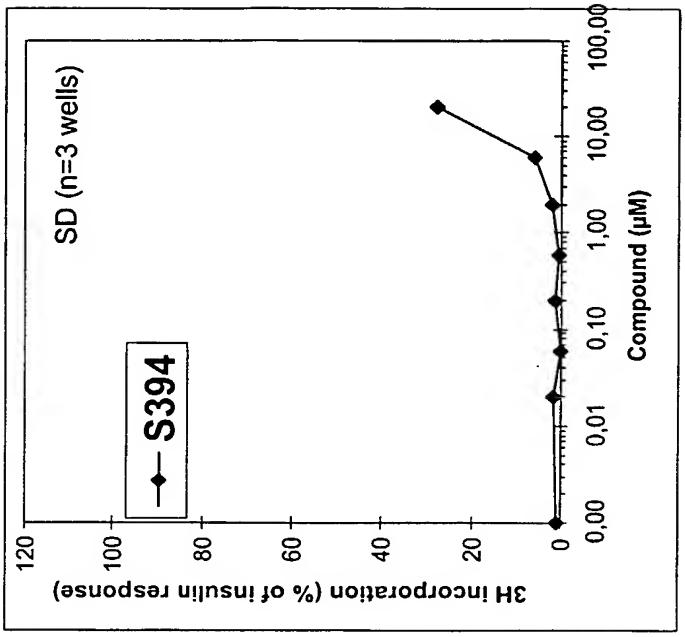


FIG. 12B



S390 = ESFYDWFERQLG
S394 = GSLDESFYDWFERQ

FIG. 12C

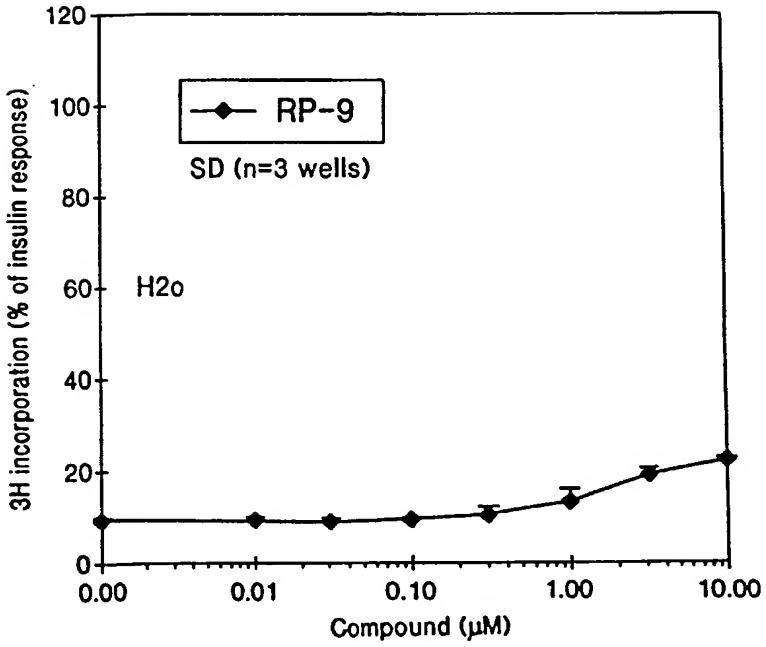

120
100
80
60
40
20
0
RP-9
SD (n=3 wells)
H2o
3H incorporation (% of insulin response)
0.00
0.01
0.10
1.00
10.00
Compound (μM)

FIG. 12D

FIG. 13A

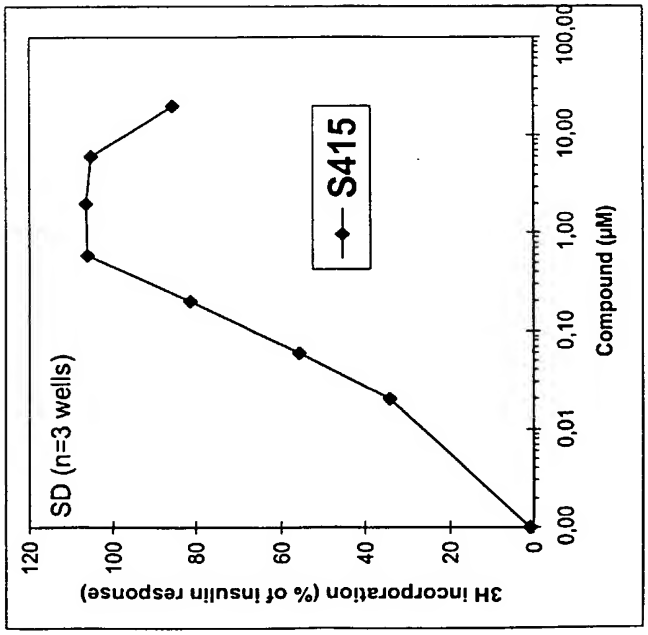
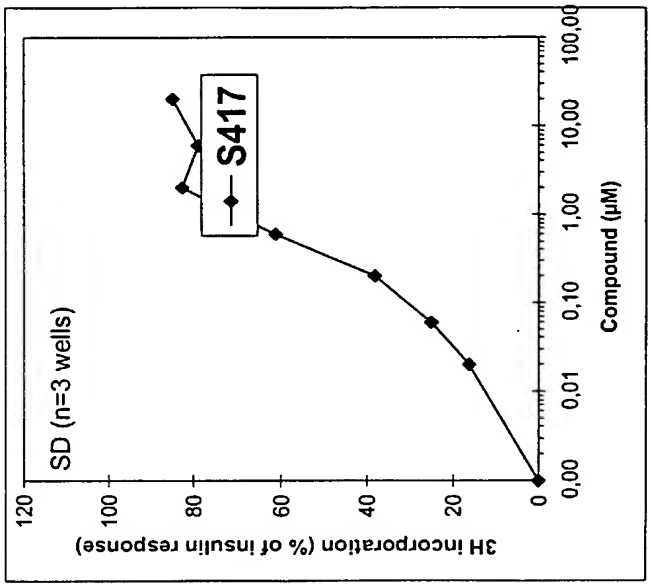


FIG. 13B



S415	$(ESFYDWFERQLGK)_2$-23
S417	23-$(ESFYDWFERQLG)_2$

FIG. 13C

FIG. 14A

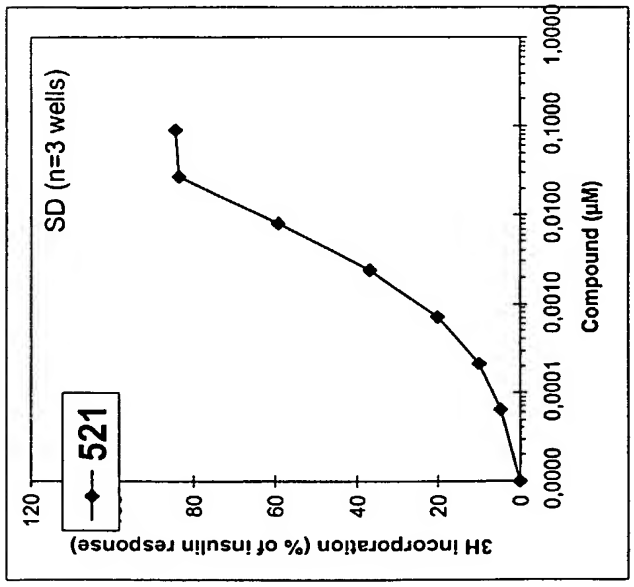
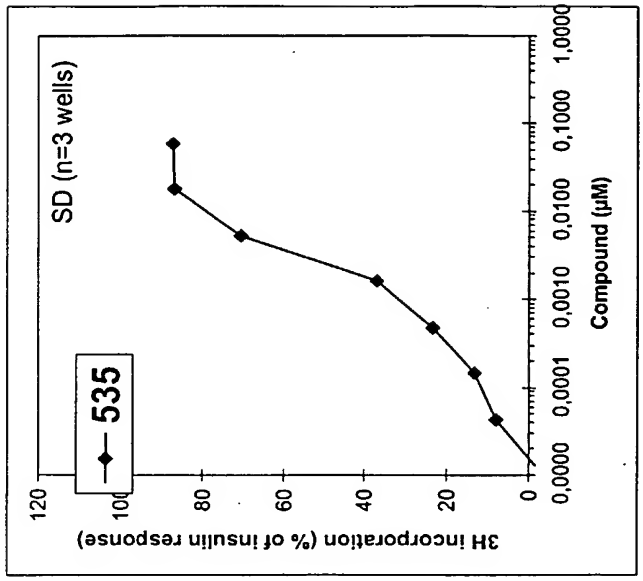


FIG. 14B



521 = RP9-6aa-RP9
535 = RP9-12aa-RP9

FIG. 14C

FIG. 15A

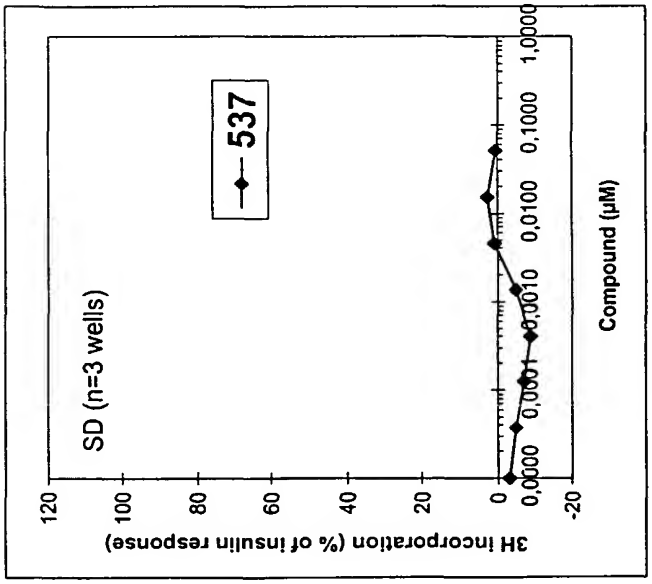
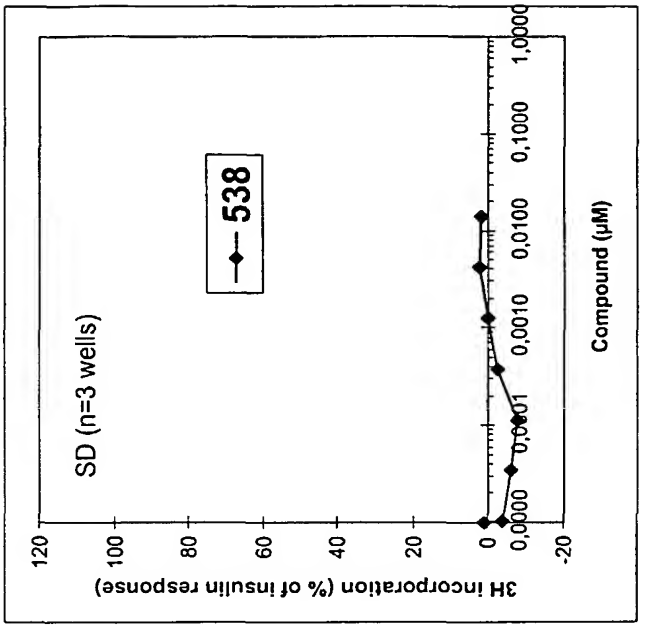


FIG. 15B



537 = RP9-6aa-D8
538 = RP9-12aa-D8

FIG. 15C

FIG. 16A

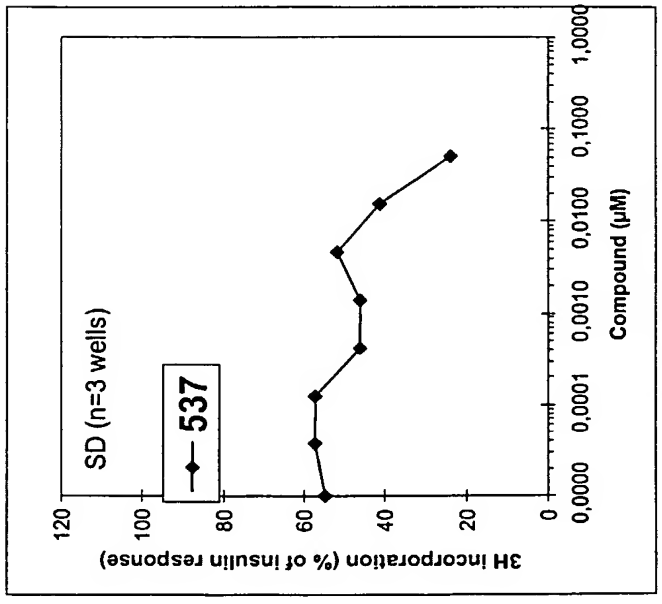
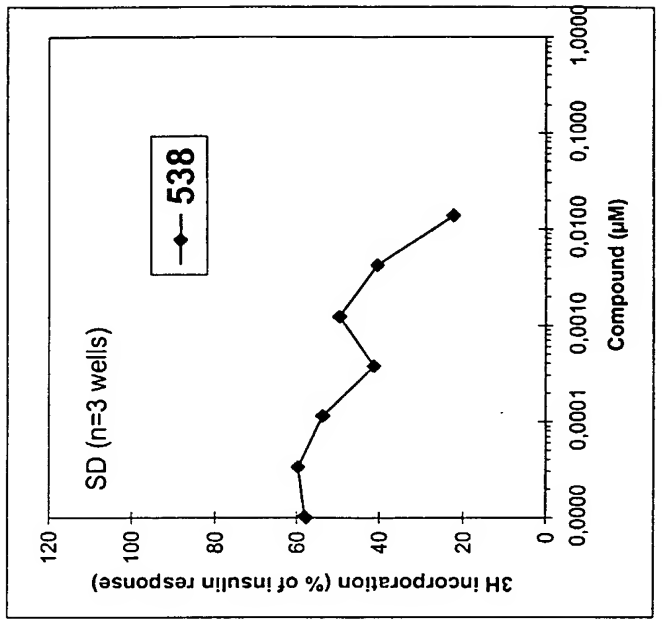


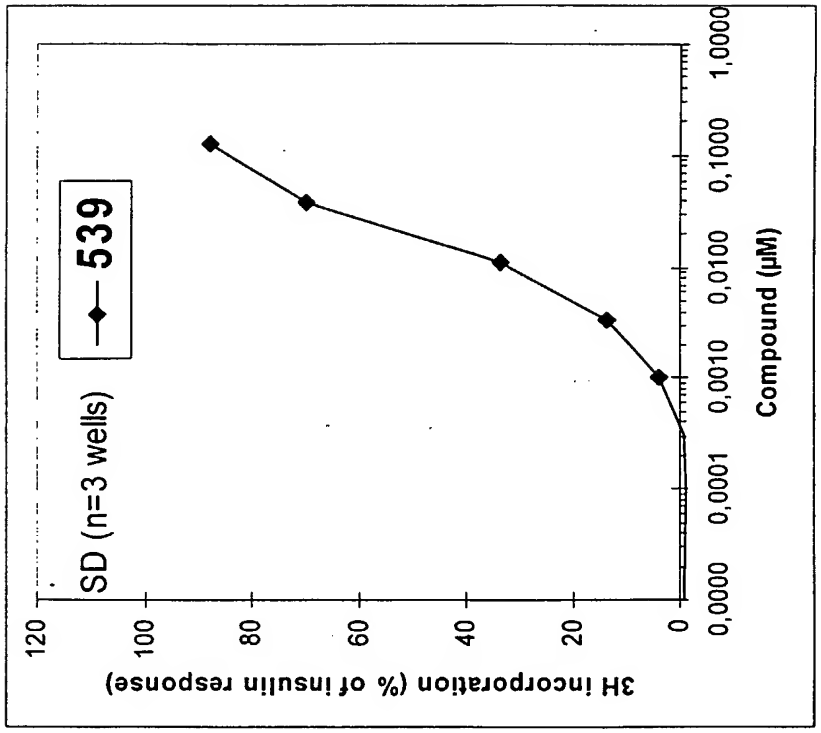
FIG. 16B



537 = RP9-6aa-D8
538 = RP9-12aa-D8

FIG. 16C

FIG. 17A



539 = D8-6aa-RP9

FIG. 17B

FIG. 18A

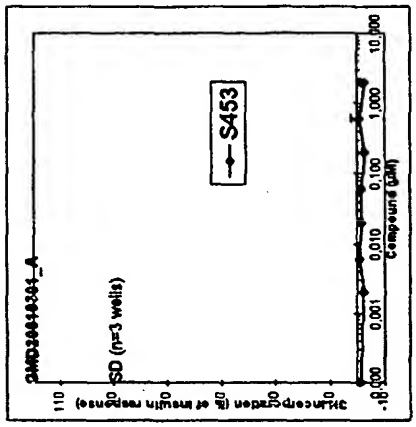


FIG. 18B

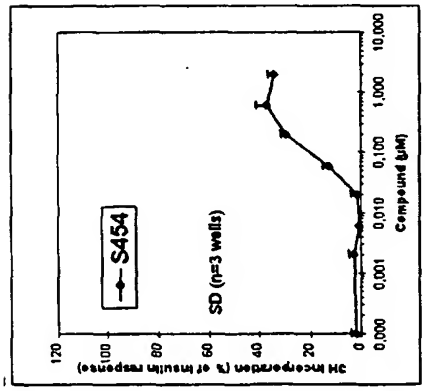


FIG. 18C

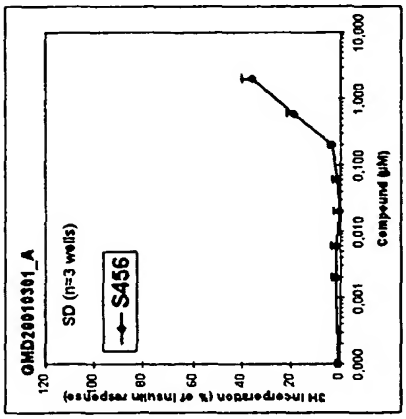


FIG. 18D

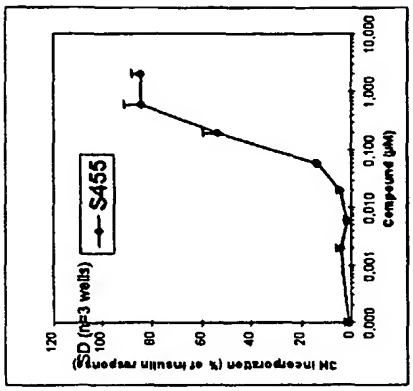


FIG. 19A

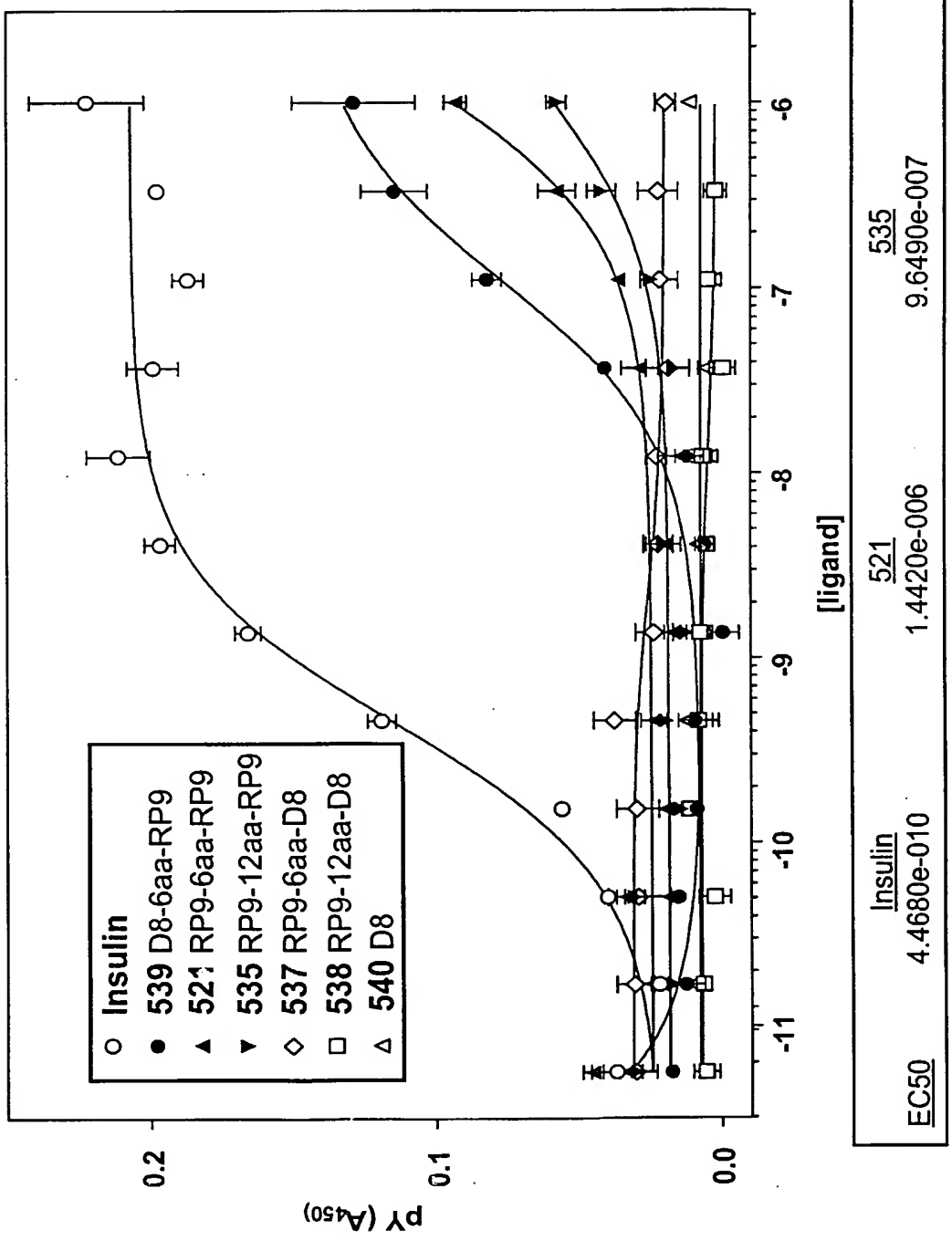
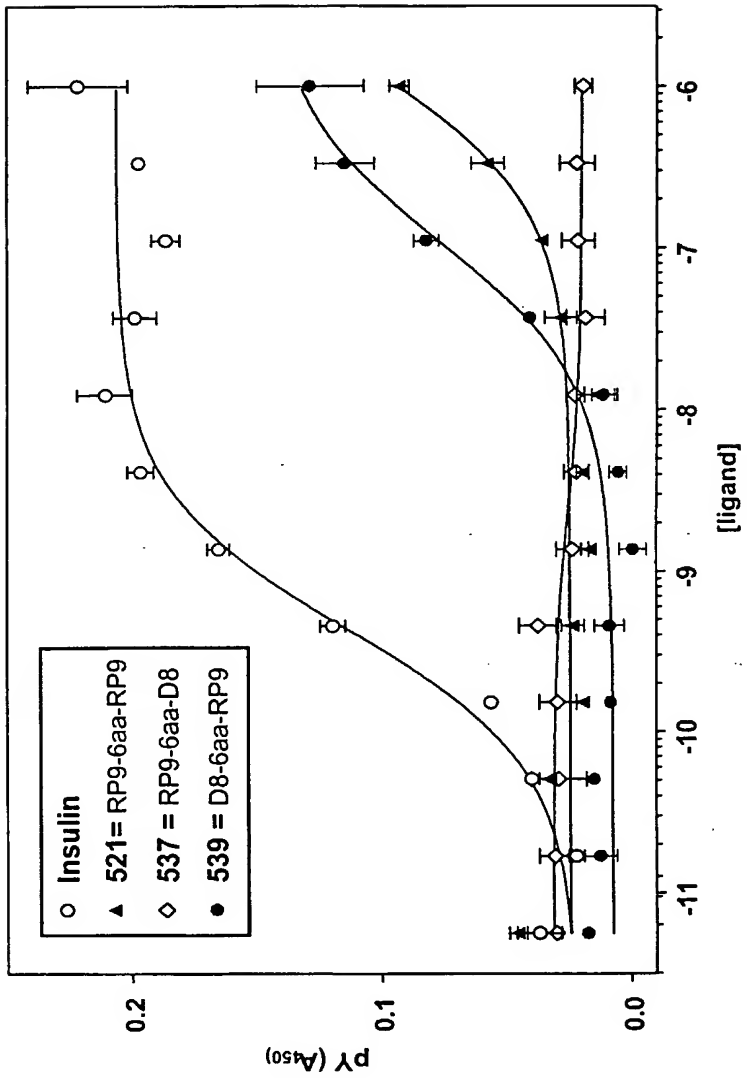


FIG. 19B

FIG. 20A



	Insulin	521	539
EC50	4.4690e-010	1.4420e-006	1.1000e-007

FIG. 20B

FIG. 21A

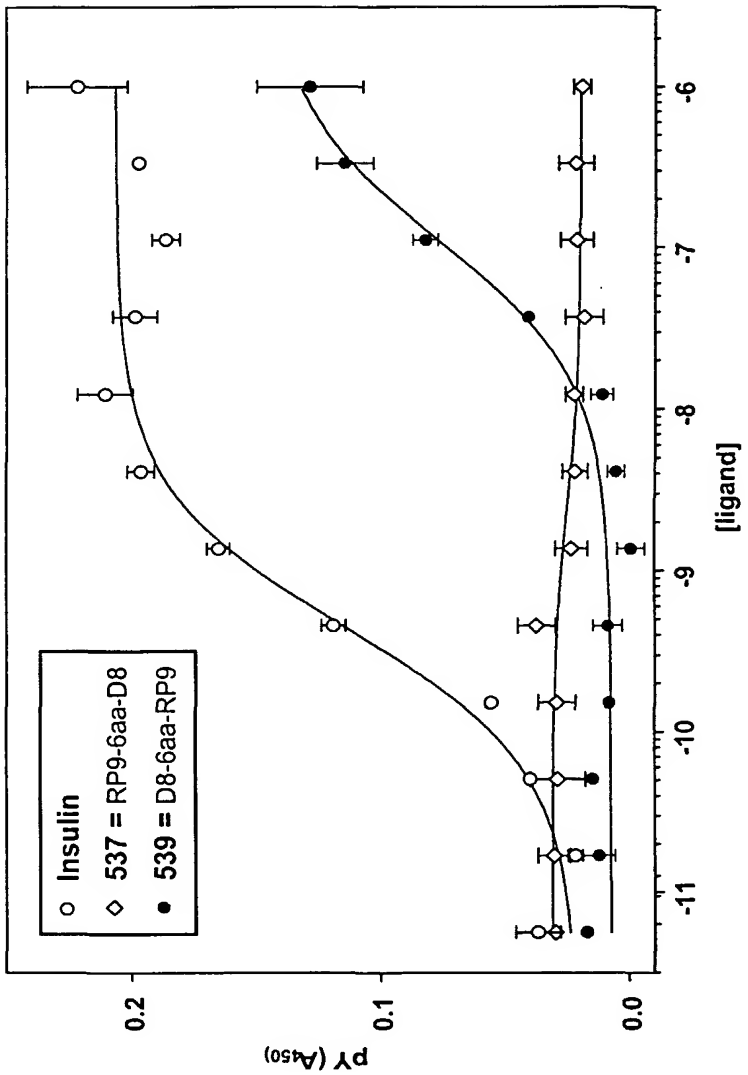
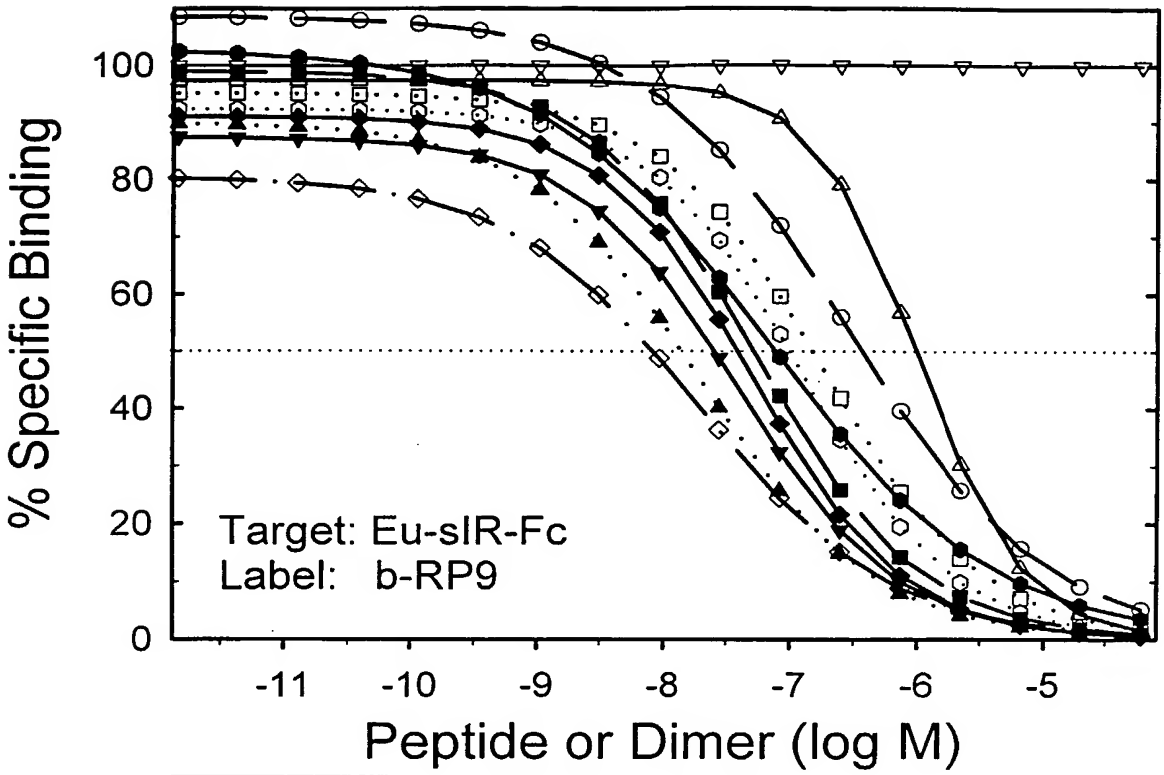


FIG. 21B

	Insulin	539
EC50	4.4680e-010	1.1000e-007

FIG. 22A



Symbol	Peptide or Dimer	Sequence
△	H2C	FHENFYDWFVQRVSKK
□	S291	(Lig-GGG-H2C)$_2$-9
▼	RP9	GSLDESFYDWFERQLGKK
■	S375	(RP9-Lig)-14-(RP9-Lig)
◇	S337	(RP9-Lig)$_2$-23
○	S391	truncated-(-GSLDE)RP9(-KK)
⬢	S390	truncated(-GSLD)RP9(-KK)
⬡	S414	(truncated(-GSLD)RP9(-KK))$_2$-14
◆	S175	GRVDWLQRNANFYDWFVAELG
▲	S380	(EE-short-S175-Lig)$_2$-9
▽	Linker 23	

FIG. 22B

FIG. 23A

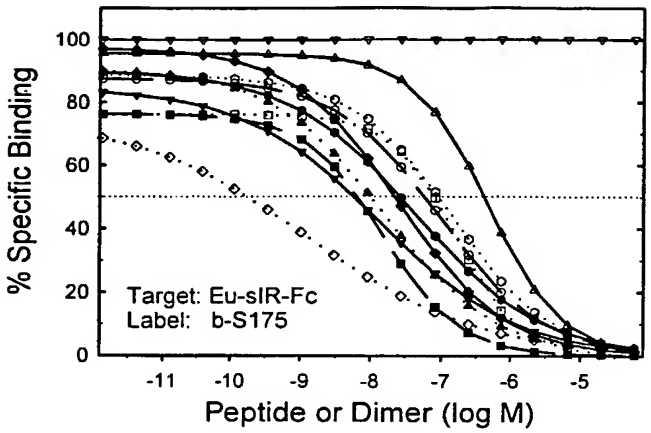
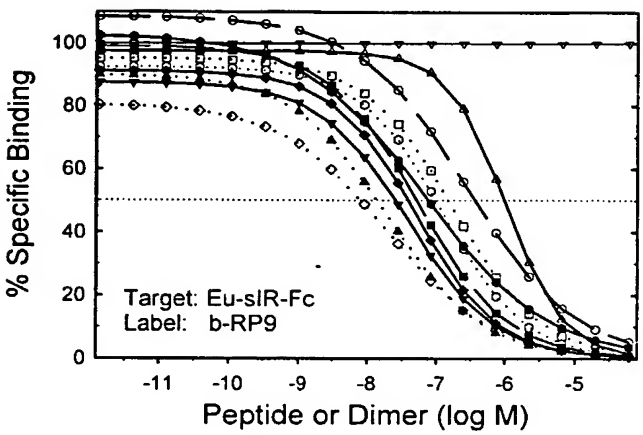


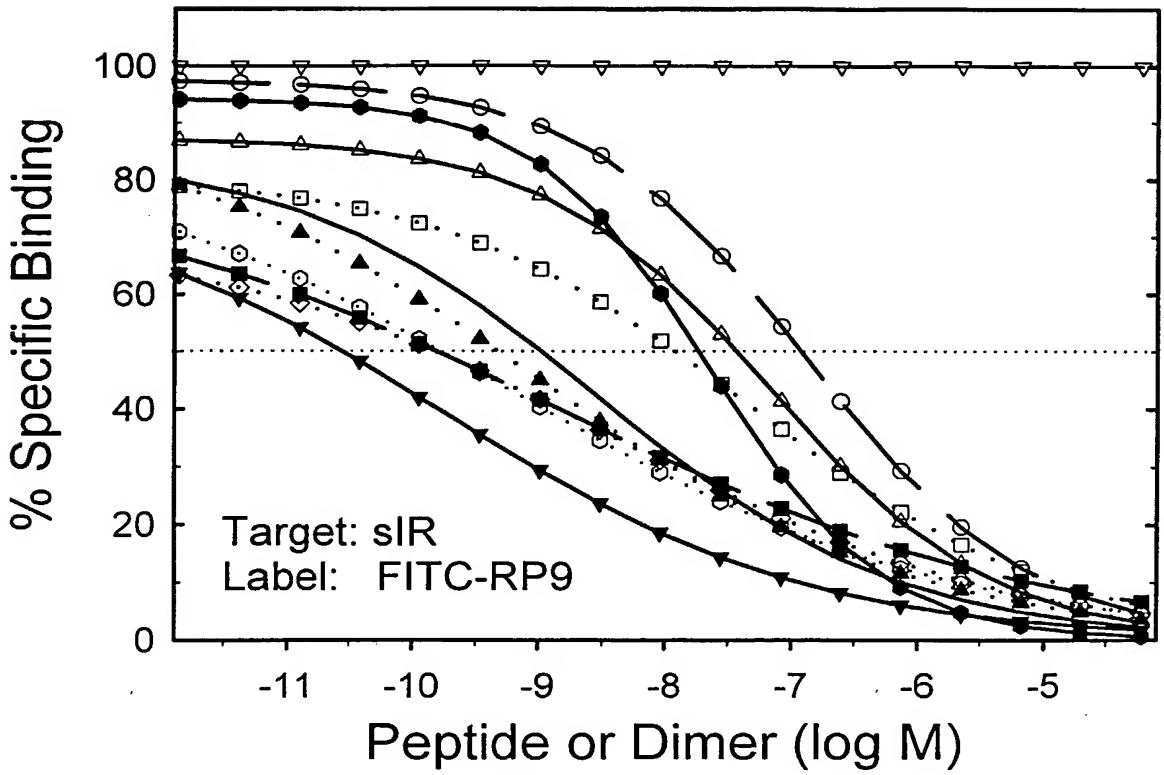
FIG. 23B



Symbol	Peptide or Dimer	Sequence
△	H2C	FHENFYDWFVQRVSKK
□	S291	(Lig-GGG-H2C)$_2$-9
▼	RP9	GSLDESFYDWFERQLGKK
■	S375	(RP9-Lig)-14-(RP9-Lig)
◇	S337	(RP9-Lig)$_2$-23
○	S391	truncated-(-GSLDE)RP9(-KK)
⬢	S390	truncated(-GSLD)RP9(-KK)
⬡	S414	(truncated(-GSLD)RP9(-KK))$_2$-14
◆	S175	GRVDWLQRNANFYDWFVAELG
▲	S380	(EE-short-S175-Lig)$_2$-9
▽	Linker 23	

FIG. 23C

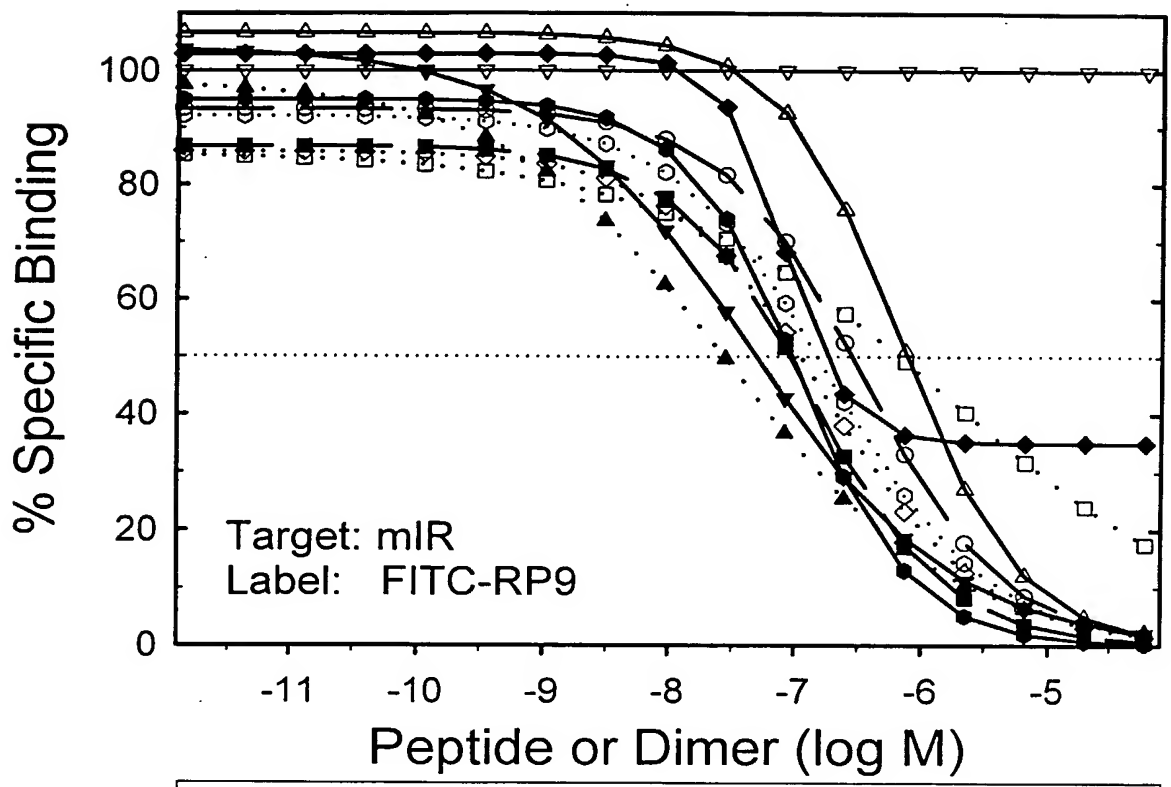
FIG. 24A



Symbol	Peptide or Dimer	Sequence
△	H2C	FHENFYDWFVQRVSKK
□	S291	(Lig-GGG-H2C)$_2$-9
▼	RP9	GSLDESFYDWFERQLGKK
■	S375	(RP9-Lig)-14-(RP9-Lig)
◇	S337	(RP9-Lig)$_2$-23
○	S391	truncated-(-GSLDE)RP9(-KK)
●	S390	truncated(-GSLD)RP9(-KK)
⬡	S414	(truncated(-GSLD)RP9(-KK))$_2$-14
◆	S175	GRVDWLQRNANFYDWFVAELG
▲	S380	(EE-short-S175-Lig)$_2$-9
▽	Linker 23	

FIG. 24B

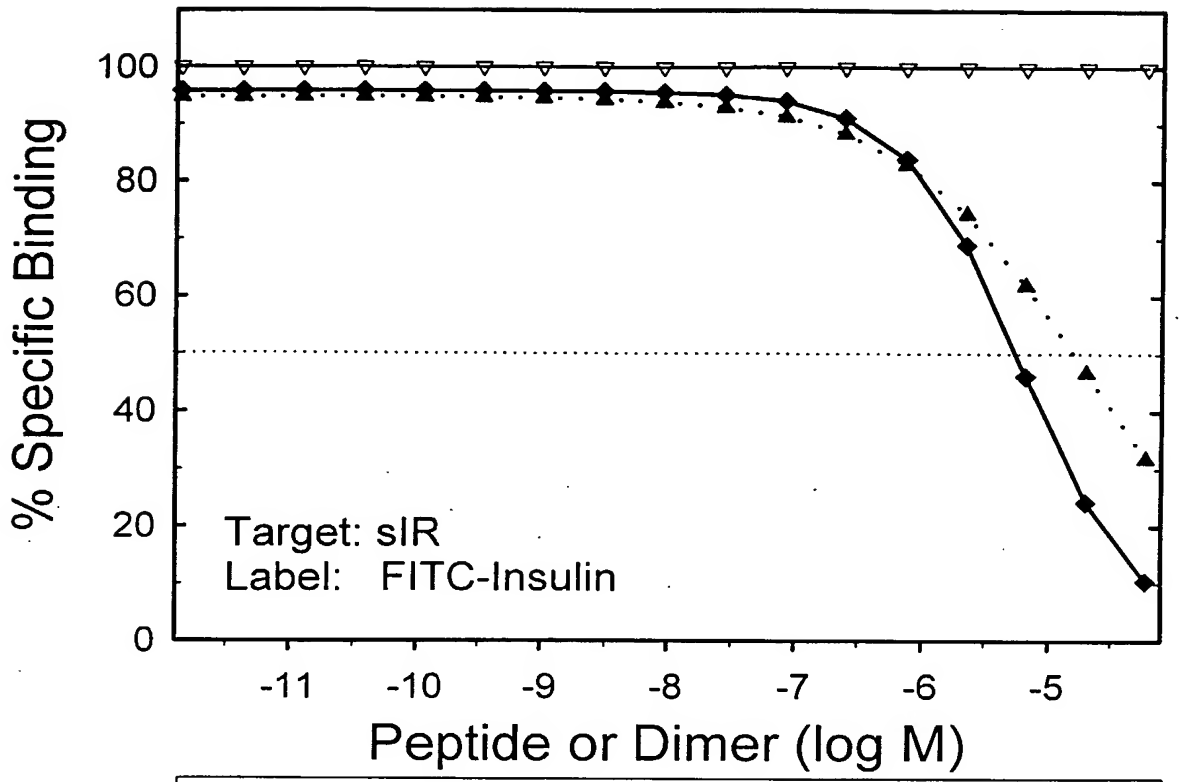
FIG. 25A



Symbol	Peptide or Dimer	Sequence
△	H2C	FHENFYDWFVQRVSKK
□	S291	(Lig-GGG-H2C)$_2$-9
▼	RP9	GSLDESFYDWFERQLGKK
■	S375	(RP9-Lig)-14-(RP9-Lig)
◇	S337	(RP9-Lig)$_2$-23
○	S391	truncated-(-GSLDE)RP9(-KK)
⬢	S390	truncated(-GSLD)RP9(-KK)
○	S414	(truncated(-GSLD)RP9(-KK))$_2$-14
◆	S175	GRVDWLQRNANFYDWFVAELG
▲	S380	(EE-short-S175-Lig)$_2$-9
▽	Linker 23	

FIG. 25B

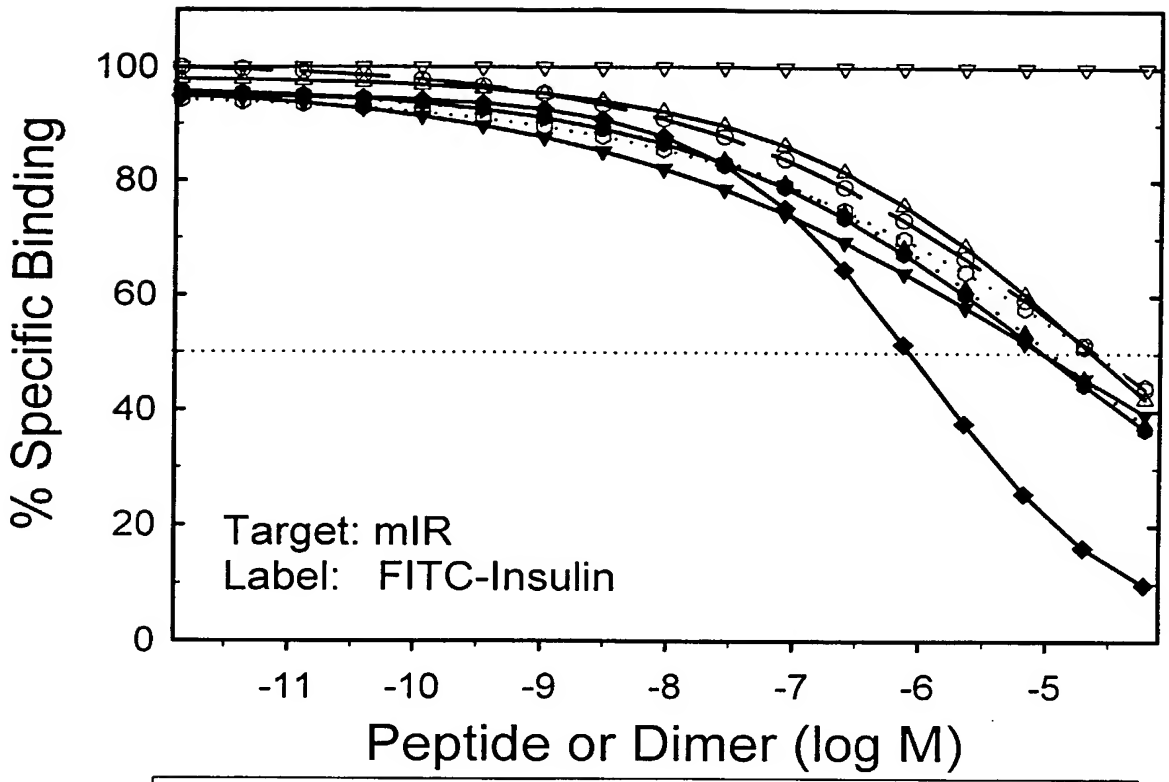
FIG. 26A



Symbol	Peptide or Dimer	Sequence
△	H2C	FHENFYDWFVQRVSKK
□	S291	(Lig-GGG-H2C)$_2$-9
▼	RP9	GSLDESFYDWFERQLGKK
■	S375	(RP9-Lig)-14-(RP9-Lig)
◇	S337	(RP9-Lig)$_2$-23
○	S391	truncated-(-GSLDE)RP9(-KK)
⬢	S390	truncated(-GSLD)RP9(-KK)
⬡	S414	(truncated(-GSLD)RP9(-KK))$_2$-14
◆	S175	GRVDWLQRNANFYDWFVAELG
▲	S380	(EE-short-S175-Lig)$_2$-9
▽	Linker 23	

FIG. 26B

FIG. 27A



Symbol	Peptide or Dimer	Sequence
△	H2C	FHENFYDWFVQRVSKK
□	S291	(Lig-GGG-H2C)$_2$-9
▼	RP9	GSLDESFYDWFERQLGKK
■	S375	(RP9-Lig)-14-(RP9-Lig)
◇	S337	(RP9-Lig)$_2$-23
○	S391	truncated-(-GSLDE)RP9(-KK)
⬢	S390	truncated(-GSLD)RP9(-KK)
⬡	S414	(truncated(-GSLD)RP9(-KK))$_2$-14
◆	S175	GRVDWLQRNANFYDWFVAELG
▲	S380	(EE-short-S175-Lig)$_2$-9
▽	Linker 23	

FIG. 27B

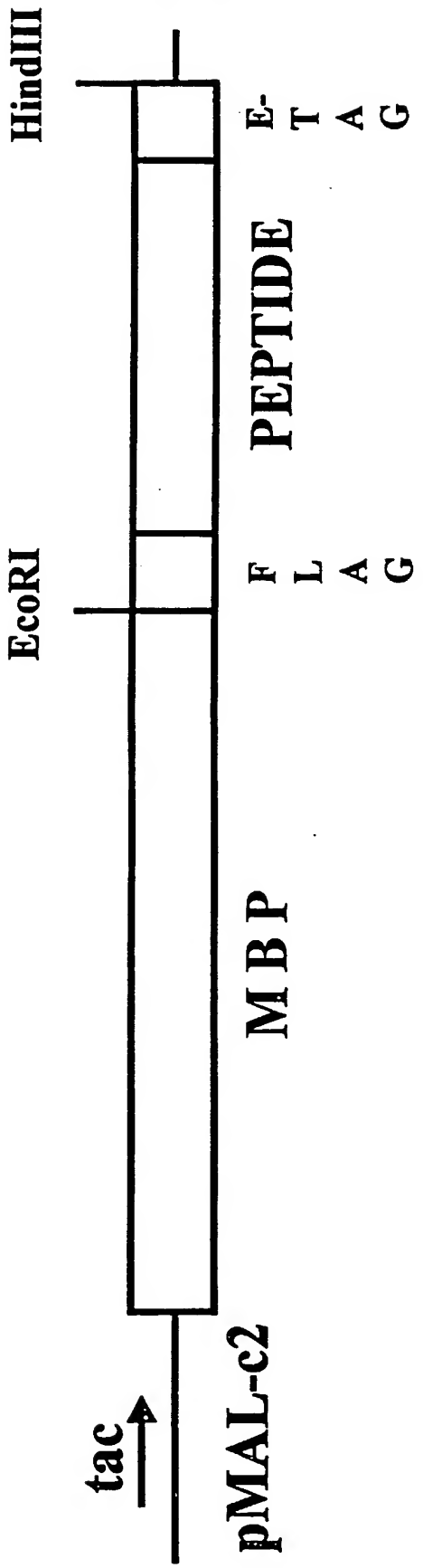


FIG. 28

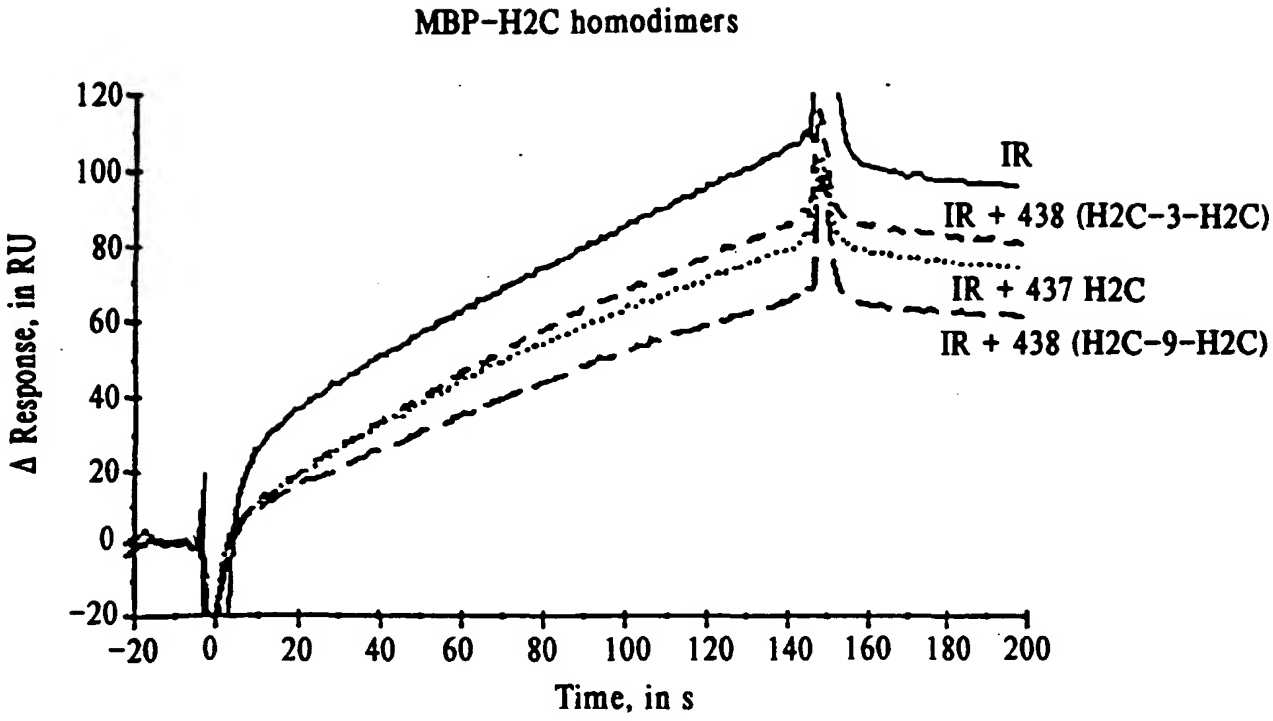

MBP-H2C homodimers
Δ Response, in RU
120
100
80
60
40
20
0
-20
-20
0
20
40
60
80
100
120
140
160
180
200
Time, in s
IR
IR + 438 (H2C-3-H2C)
IR + 437 H2C
IR + 438 (H2C-9-H2C)

FIG. 29

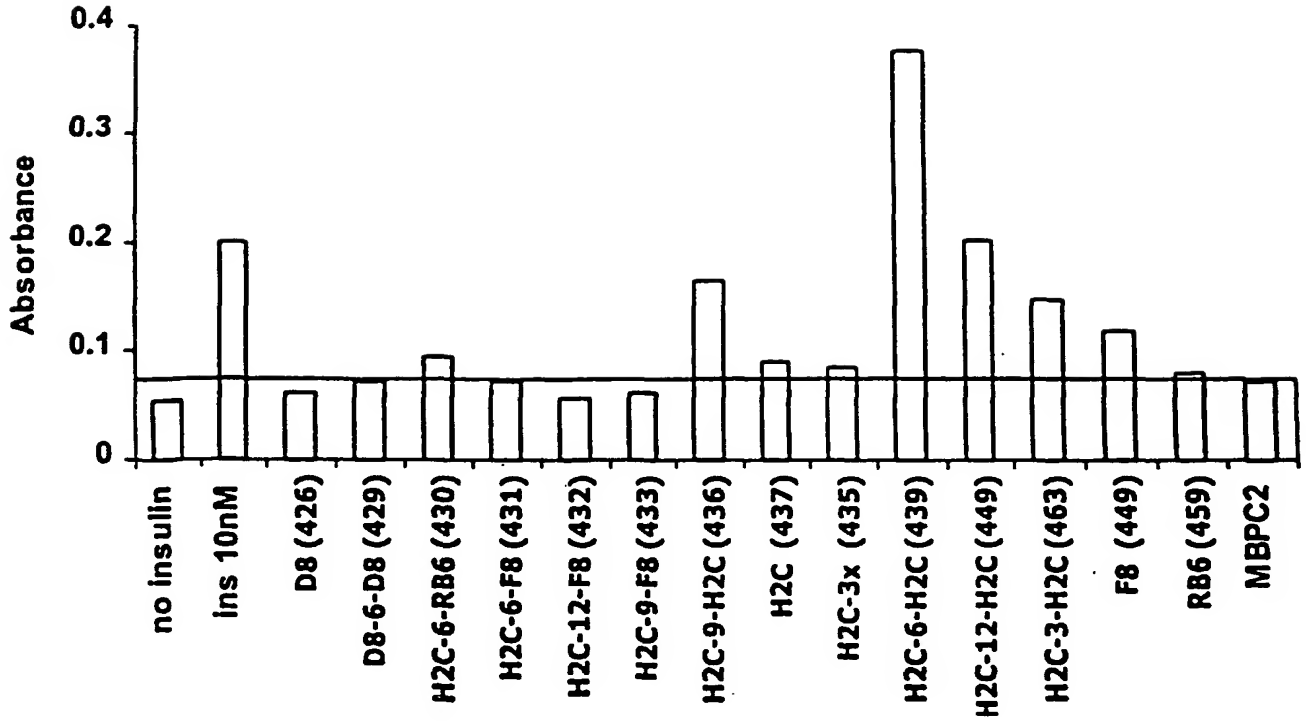

Absorbance
0.4
0.3
0.2
0.1
0
no insulin
Ins 10nM
D8 (426)
D8-6-D8 (429)
H2C-6-RB6 (430)
H2C-6-F8 (431)
H2C-12-F8 (432)
H2C-9-F8 (433)
H2C-9-H2C (436)
H2C (437)
H2C-3x (435)
H2C-6-H2C (439)
H2C-12-H2C (449)
H2C-3-H2C (463)
F8 (449)
RB6 (459)
MBPC2

FIG. 30

FIG. 31A

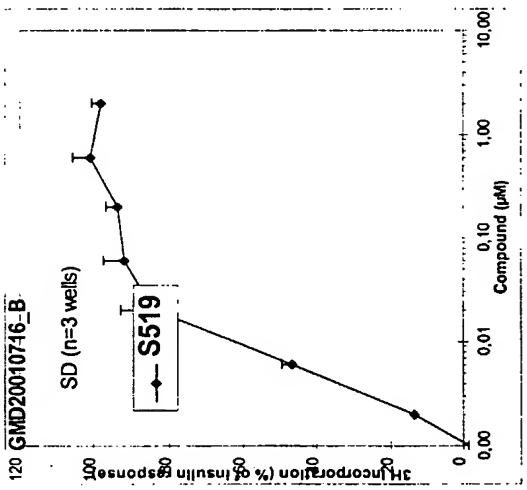
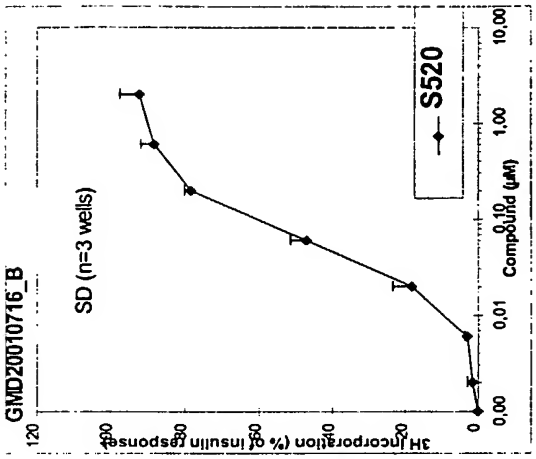


FIG. 31B

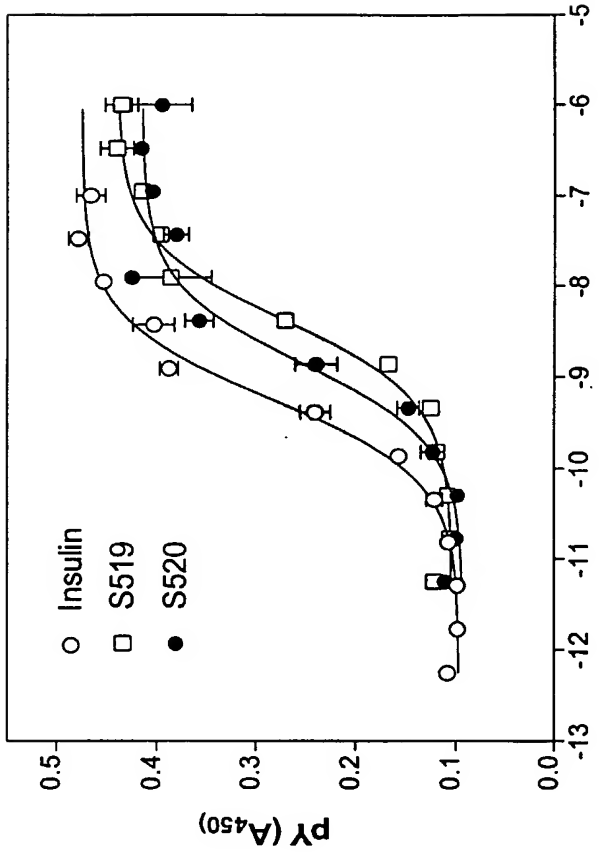


EC_{50}

- Insulin: 0.050 nM
- S519: 4.19 nM
- S520: 58.8 nM

FIG. 31C

FIG. 32A



	Insulin	S519	S520
Equation 1			
Best-fit values			
BOTTOM	0.09614	0.1038	0.09202
TOP	0.4740	0.4388	0.4145
LOGEC50	-9.237	-8.380	-8.852
EC50	5.8000e-010	4.1660e-009	1.4060e-009

FIG. 32B

FIG. 33

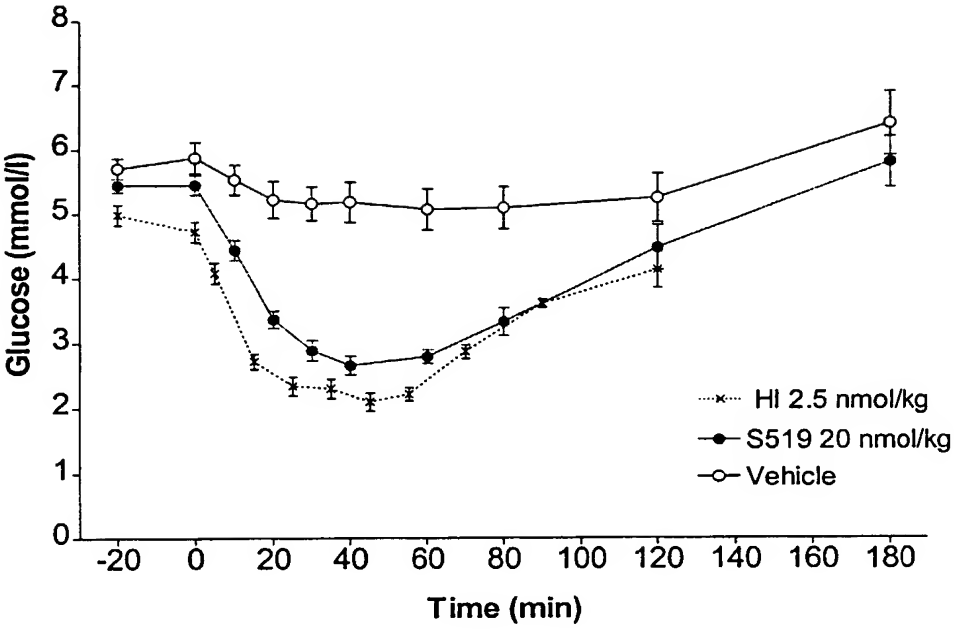

Glucose (mmol/l)
8
7
6
5
4
3
2
1
0
-20
0
20
40
60
80
100
120
140
160
180
Time (min)
HI 2.5 nmol/kg
S519 20 nmol/kg
Vehicle

FIG. 34A

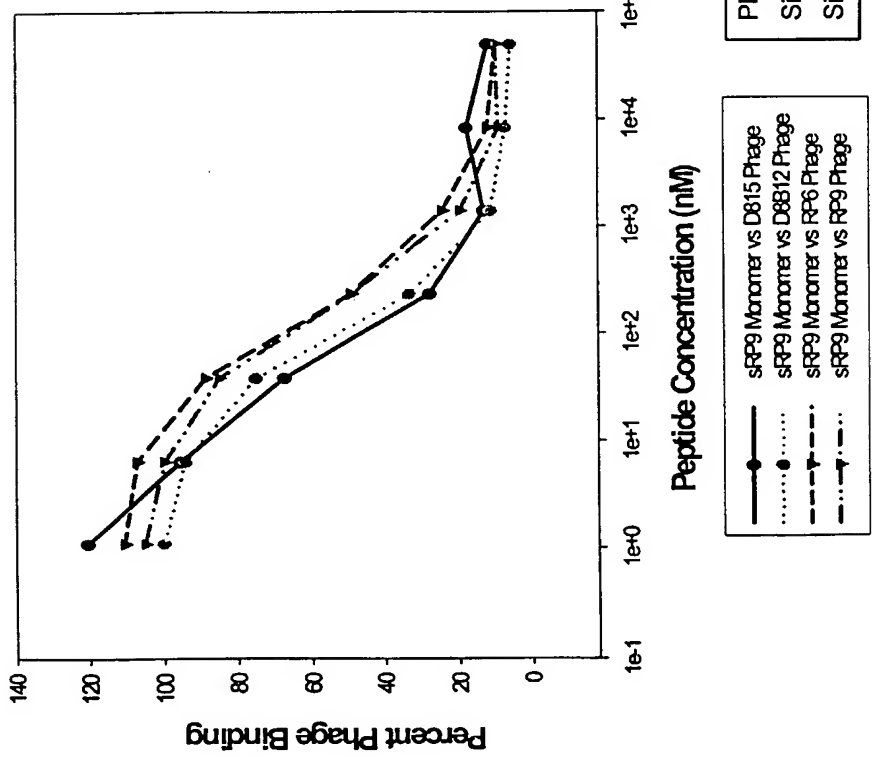


FIG. 34B

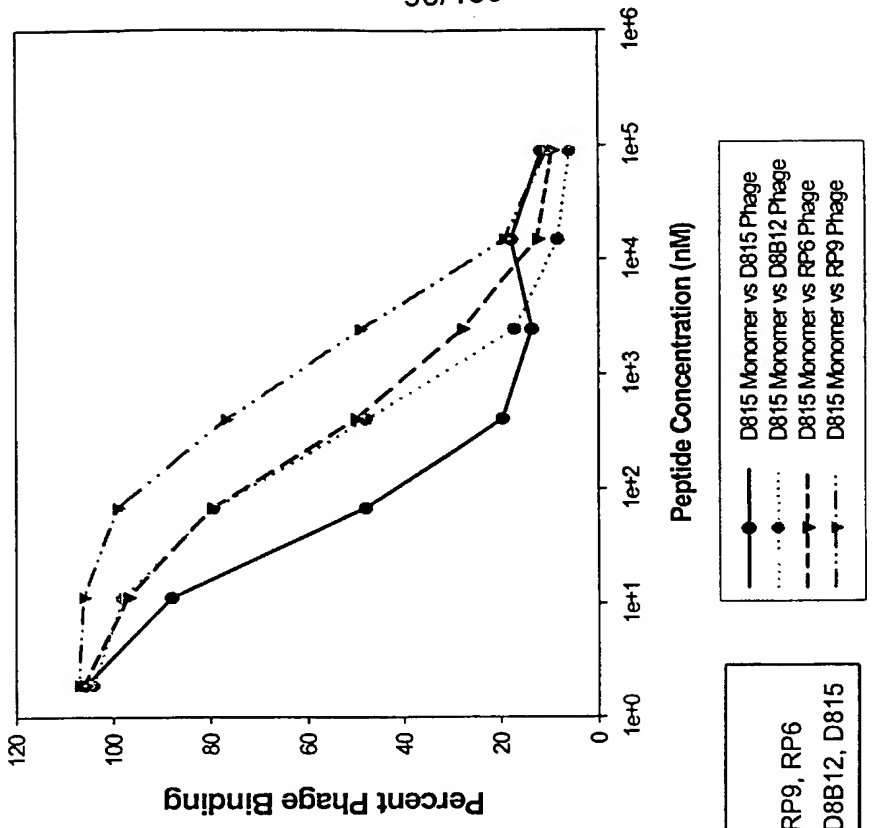


FIG. 34C

FIG. 34D

FIG. 34E

FIG. 35A

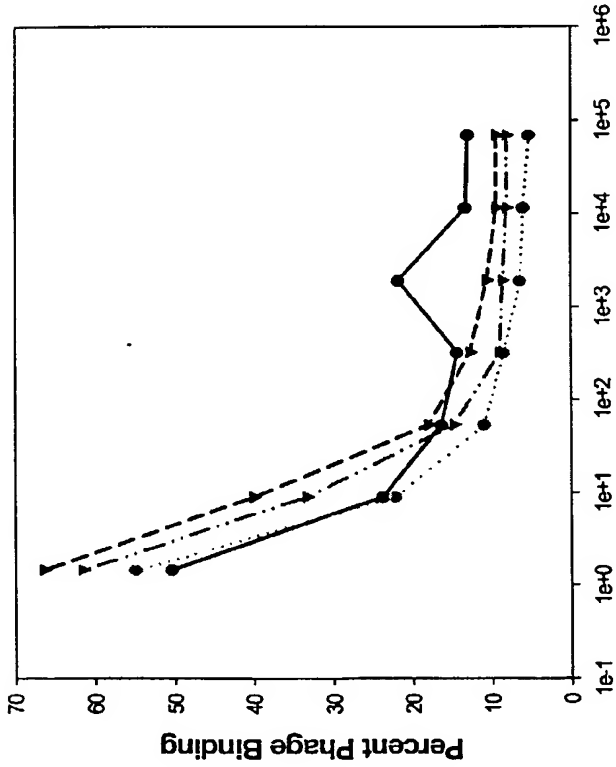


FIG. 35B

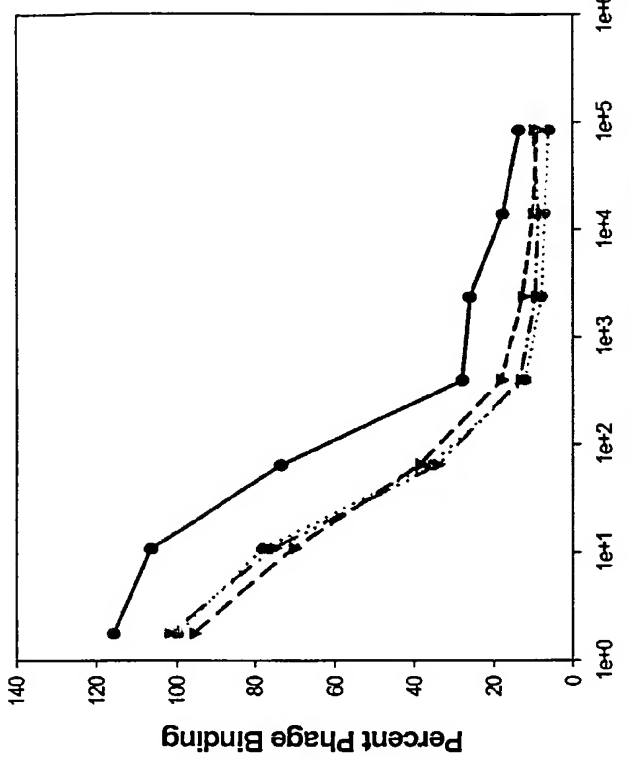


FIG. 35C

Phage:
Site 1: RP9, RP6
Site 2: D8B12, D815

FIG. 35D

FIG. 35E

FIG. 36

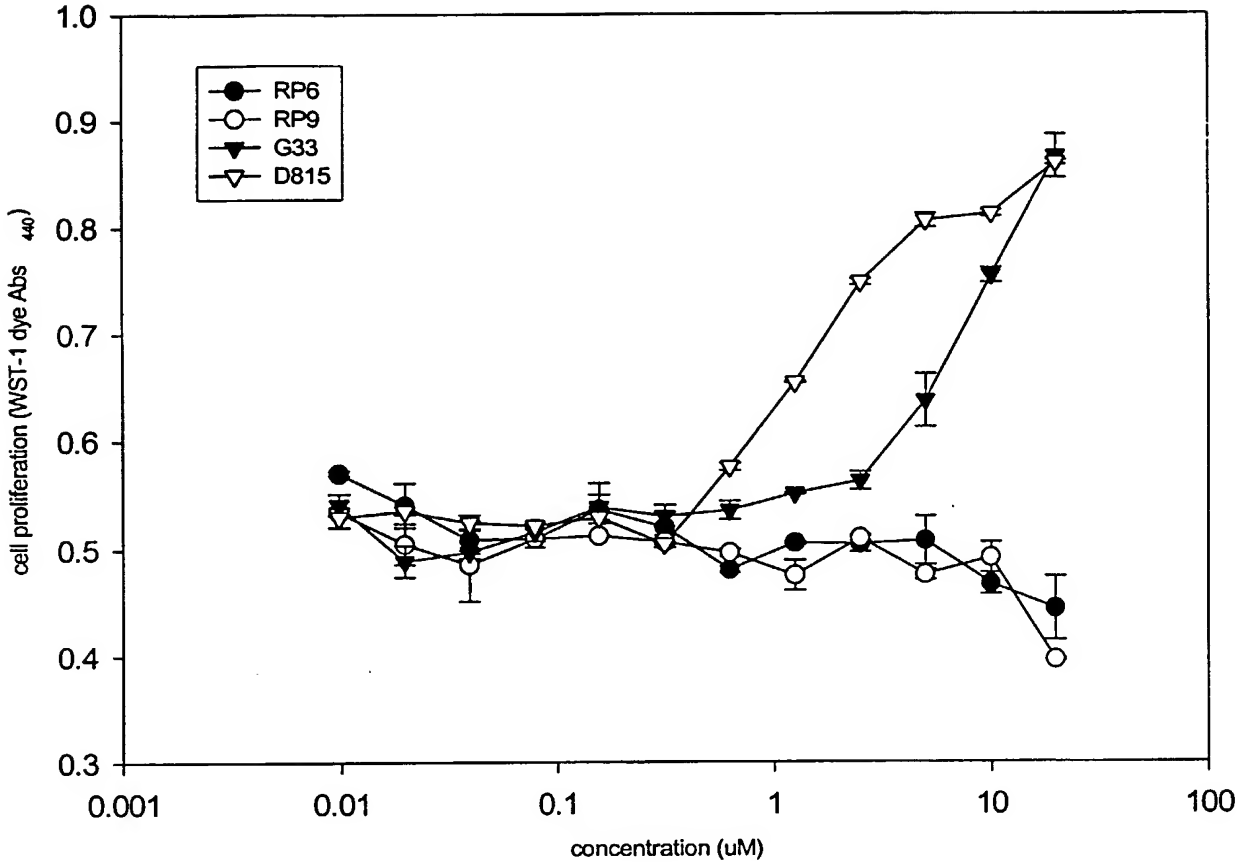

RP6
RP9
G33
D815
cell proliferation (WST-1 dye Abs 440)
concentration (uM)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.001
0.01
0.1
1
10
100

FIG. 37

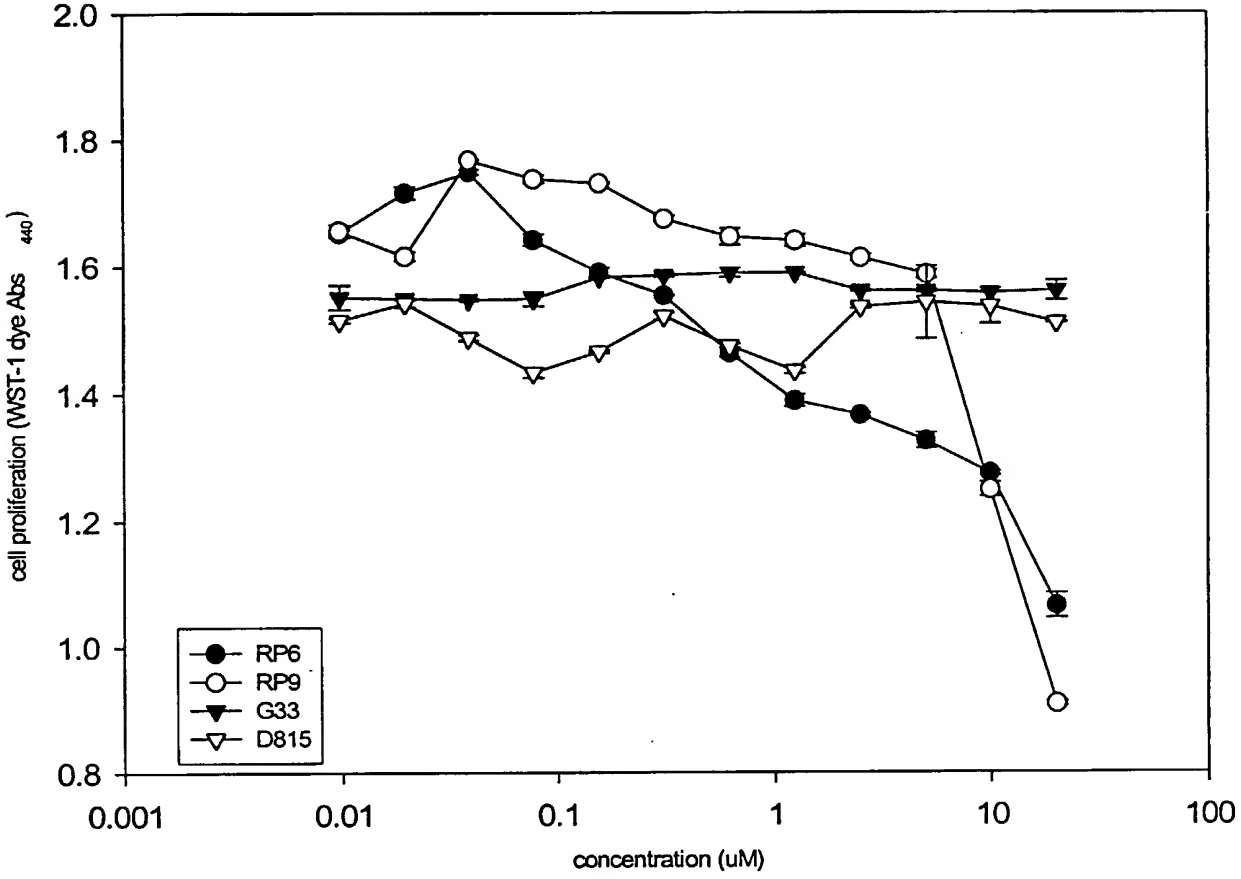
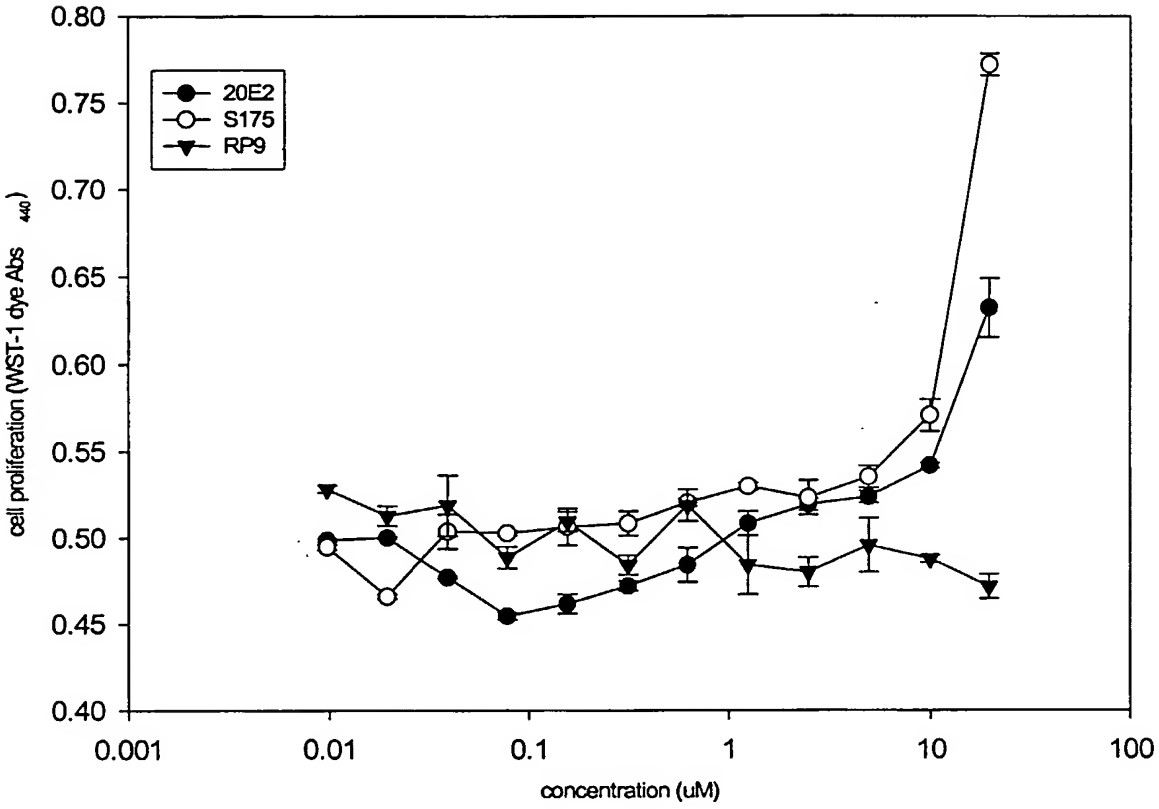

2.0
1.8
1.6
1.4
1.2
1.0
0.8
cell proliferation (WST-1 dye Abs 440)
RP6
RP9
G33
D815
0.001
0.01
0.1
1
10
100
concentration (uM)

FIG. 38


0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
cell proliferation (WST-1 dye Abs 440)
20E2
S175
RP9
0.001
0.01
0.1
1
10
100
concentration (uM)

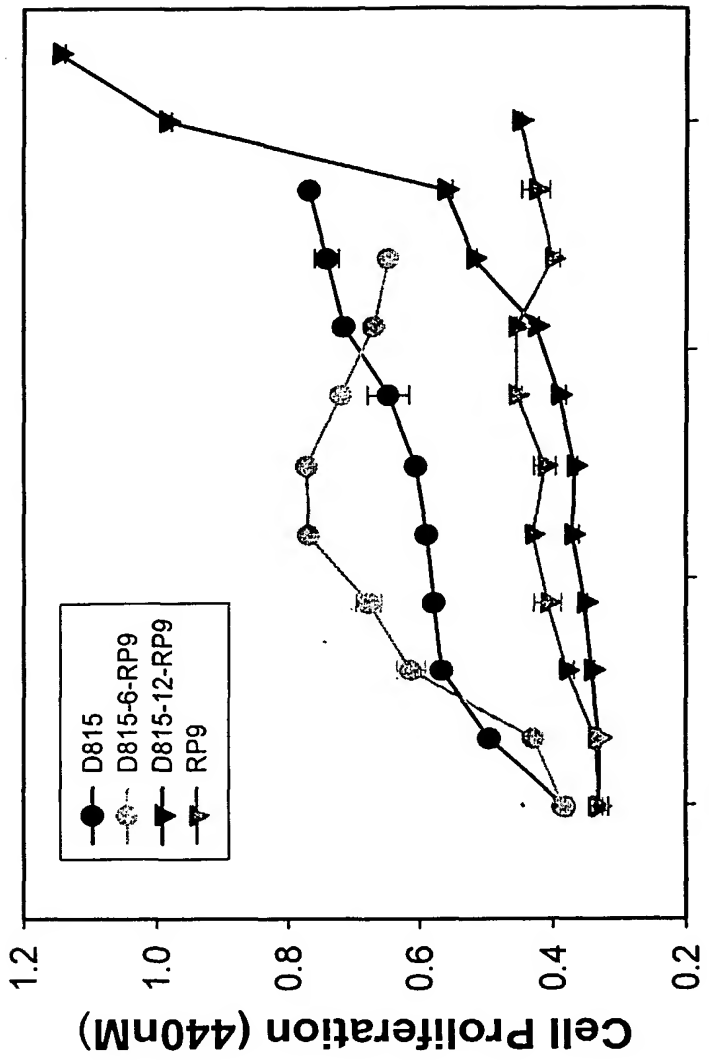

Cell Proliferation (440nM)
1.2
1.0
0.8
0.6
0.4
0.2
-8
-7
-6
-5
Log [Peptide or Dimer] (M)
D815
D815-6-RP9
D815-12-RP9
RP9

FIG. 39

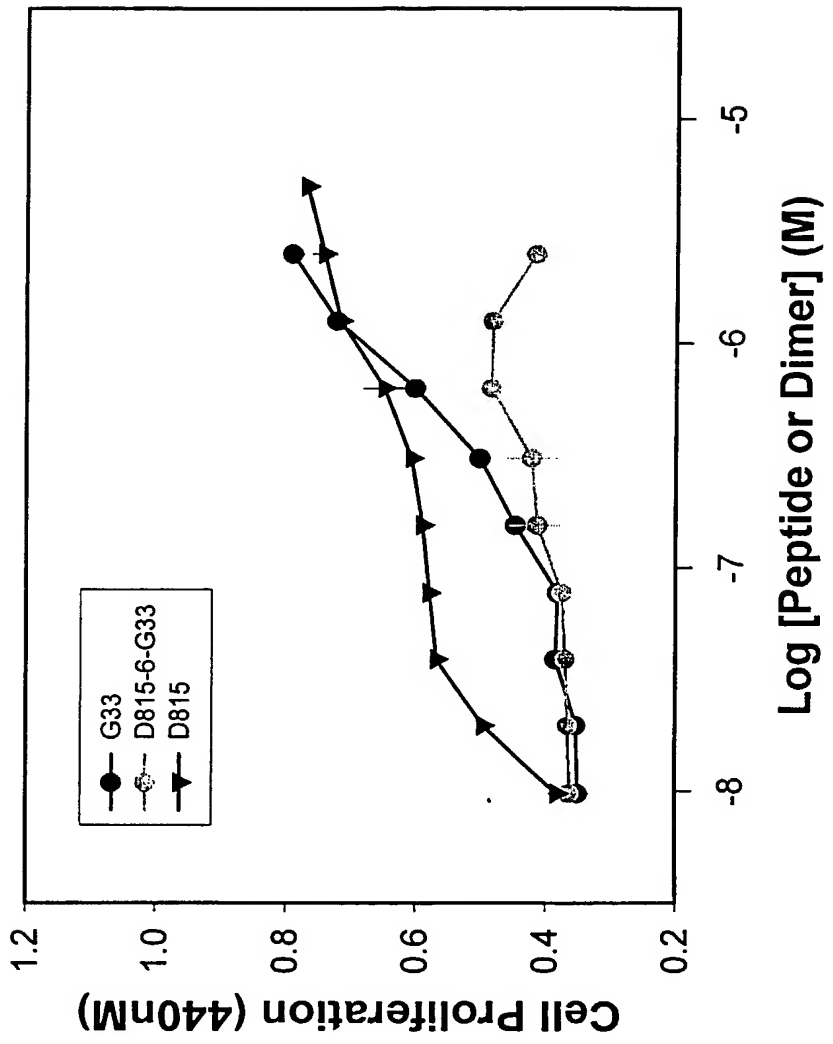

Cell Proliferation (440nM)
1.2
1.0
0.8
0.6
0.4
0.2
-8
-7
-6
-5
Log [Peptide or Dimer] (M)
G33
D815-6-G33
D815

FIG. 40

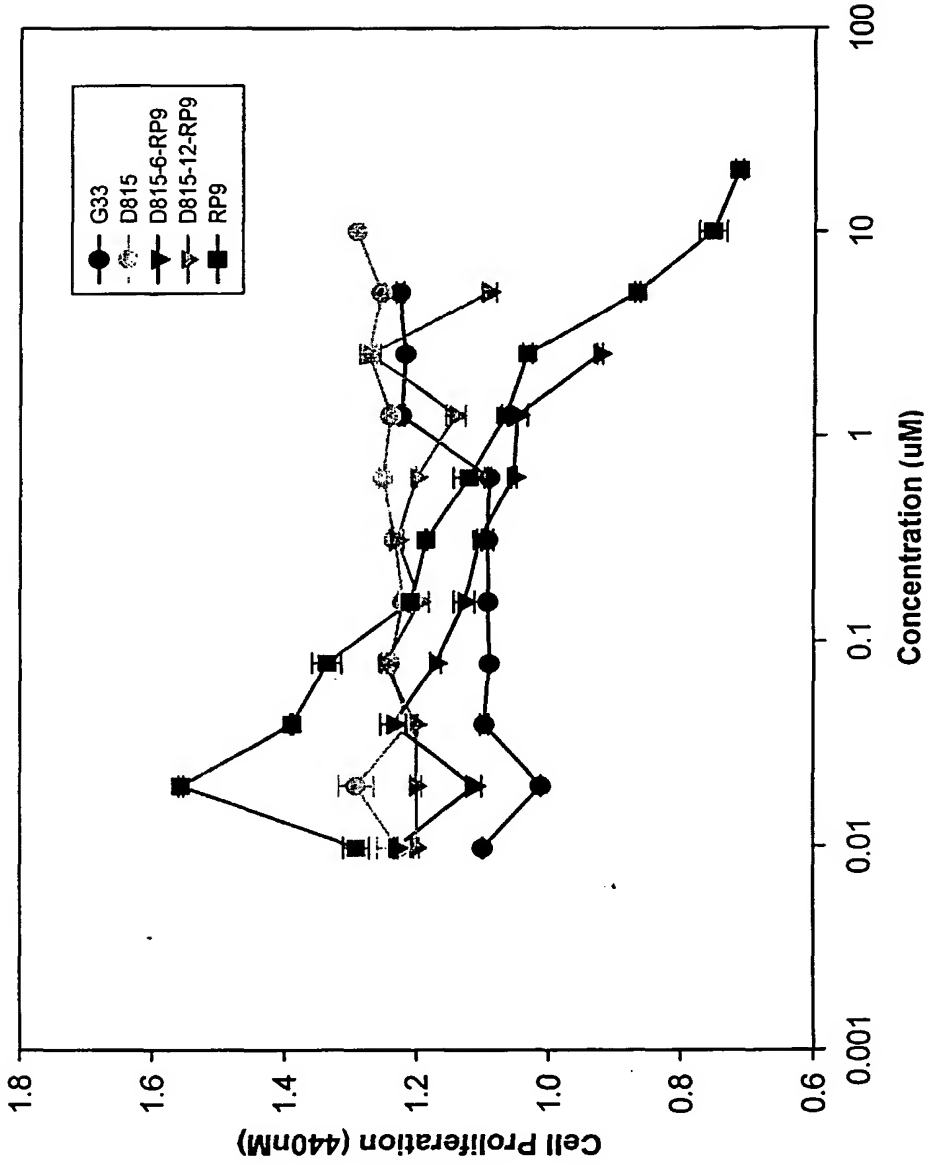
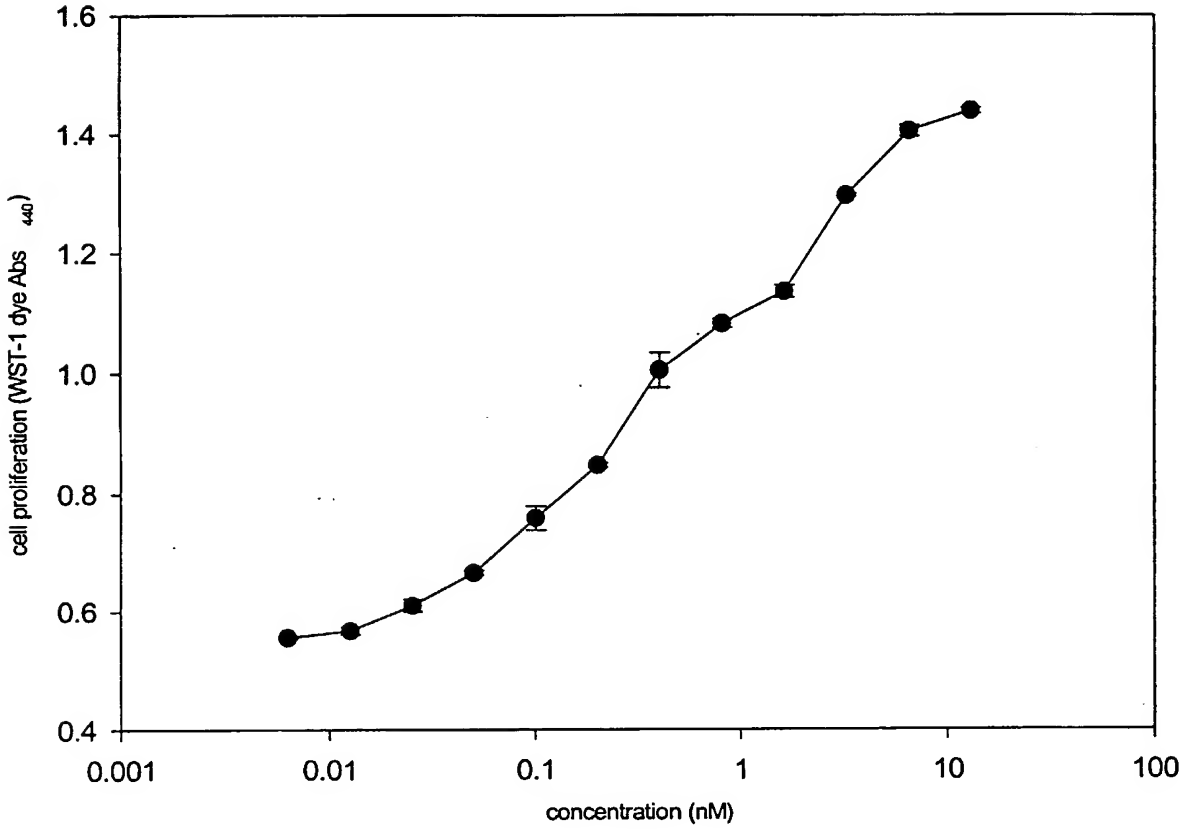

G33
D815
D815-6-RP9
D815-12-RP9
RP9
Cell Proliferation (440nM)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.001
0.01
0.1
1
10
100
Concentration (uM)

FIG. 41

FIG. 42


1.6
1.4
1.2
1.0
0.8
0.6
0.4
cell proliferation (WST-1 dye Abs 440)
0.001
0.01
0.1
1
10
100
concentration (nM)

Clone #	Sequence	Etag	IGFR	LDH	IGFR/LDH	Binder
IGFR-G33-4-A1		15.5	10.3	1.1	9.5	HIT
IGFR-G33-4-A2		4.8	2.9	1.2	2.5	HIT
IGFR-G33-4-A3		14.9	16.1	1.5	10.6	HIT
IGFR-G33-4-A4	PAMAVGYPQPCAKSTYERGRGSALESRCYQAAAGAP	8.6	5.4	1.3	4.3	HIT
IGFR-G33-4-A5		23.5	12.1	3.4	3.6	HIT
IGFR-G33-4-A6		10.8	5.2	1.2	4.4	HIT
IGFR-G33-4-A7	MC	13.3	5.0	0.8	6.2	HIT
IGFR-G33-4-A8	PAMACKVC*CCSVSCYDGFPRSGAHPGRRWAAAGAP	6.0	1.7	1.0	1.8	CAND
IGFR-G33-4-A9	PAMAFKVSLSCGESFYEWFAGLVRDPTCGWTAAGAP	10.8	6.3	1.1	5.7	HIT
IGFR-G33-4-A10	MC	6.7	8.9	2.1	4.2	HIT
IGFR-G33-4-A11	AGHGACEFQVMFG*LVHLLGFPGRLGKGLAAGA	5.8	5.8	1.2	4.9	HIT
IGFR-G33-4-A12	RPWRGSWLRLVGRRVECYCAERGATRGW*CAAAGAP	3.3	2.6	1.1	2.4	HIT
IGFR-G33-4-B1	AGHGDFGALSCKAAVVAWVPVQTAGLRVRVAAAGAP	8.1	3.2	1.2	2.6	HIT
IGFR-G33-4-B2	PAMAPRLYQGCPESFYAWTAGHVSPALYGWAAAGAP	4.8	4.1	1.1	3.8	HIT
IGFR-G33-4-B3	PGHGVSVRAGVSGMLRREVAG#CVSAWEGLCGRRCA	6.4	2.0	0.9	2.2	CAND
IGFR-G33-4-B4	PAMAGMDPQ#CTVASSRWFASPV#VVWRC#AAAGAP	5.8	5.4	2.6	2.1	HIT
IGFR-G33-4-B5	PAMAGMFSQTCPEGFYGWFAGQASDSSLCRAAAGAP	15.8	2.5	0.9	3.0	HIT
IGFR-G33-4-B6	PAMAPLGFRSCAGAY*VGCGRRVAFE#RCWAAAGAP	7.7	2.3	1.2	1.9	HIT
IGFR-G33-4-B7	PAMAGILCPSCPHFLVDS#AAQDAAGQWPSAAAGAP	7.2	3.3	1.2	2.8	HIT
IGFR-G33-4-B8	MC	4.8	1.4	1.0	1.5	
IGFR-G33-4-B9	PAMARRIPRECGDSFYVGLRWLVENPRSDWAAAGAP	6.2	1.9	1.0	1.9	CAND
IGFR-G33-4-B10	PAMADRIGVQCPDSFYGWFAVQEPGTSGGLAAAGAP	8.5	3.5	1.0	3.5	HIT
IGFR-G33-4-B11	PAMAGLPS*SCRVAMYKGQAAWSCSAAAGAP	4.9	3.9	0.9	4.3	HIT
IGFR-G33-4-B12	RPWRLILVTLVREASMTGSGVWYPRRGGAGPAEGA	3.6	2.6	1.0	2.5	HIT
IGFR-G33-4-C1		27.7	24.5	1.0	24.5	HIT
IGFR-G33-4-C2	PAMAGSARQVCVDGVVGWREG*VVDQWL#RAAAGAP	28.2	8.4	1.9	4.5	HIT
IGFR-G33-4-C3	PAMAGIMQRACEGGFTDCLWSLISGASSGRAAAGAP	29.7	5.3	1.3	4.0	HIT
IGFR-G33-4-C4	RPWRVSSLRHVRVTCGELFGGQVSELFCLCAAAGAP	7.5	5.6	1.1	5.0	HIT
IGFR-G33-4-C5		4.5	4.4	1.2	3.8	HIT
IGFR-G33-4-C6	PAMAGLIYMSCLAYFDDLIERRLEKPG#RFAAAGAP	36.1	22.9	6.3	3.7	HIT
IGFR-G33-4-C7	PAMAGIMPQSCGETSGKCMRGQVSLRWRWSAAAGAP	10.0	1.7	1.1	1.6	CAND
IGFR-G33-4-C8	PAMAFILPRSCEDYLYDFLASKVVHVFRSLAAAGAP	9.7	6.9	1.9	3.6	HIT
IGFR-G33-4-C9	PAMACMSSQPCGESFYDWFAGQVRDPGWESAAAGA	23.3	19.4	9.5	2.1	HIT
IGFR-G33-4-C10	RPWRGWAIRGVRHRC*GAWRGQVAQELCR#AAAGA	30.2	9.3	4.3	2.2	HIT

FIG. 43A-1

Clone #	Sequence	Etag	IGFR	LDH	IGFR/LDH	Binder
IGFR-G33-4-C11	PAMAGIASHTCPGGFYEWFACQSRAPGWDGAAAGAP	10.6	6.7	1.1	6.1	HIT
IGFR-G33-4-C12		19.2	30.2	5.2	5.9	HIT
IGFR-G33-4-D1	PAMAGRIARACPDSMFGWLAGQGSQQSGWQAAAGAP	2.6	1.8	1.1	1.7	CAND
IGFR-G33-4-D2	PAMARPISPLC#RRSKDEDASRVSLPGFFCAAAGAP	6.2	5.1	1.2	4.4	HIT
IGFR-G33-4-D3	MC	31.0	8.5	1.0	8.3	HIT
IGFR-G33-4-D4	PAMADYKDDDDKTFYACLASLMAGTPRQYRTPWARCPAAAGAP	4.8	1.7	1.1	1.5	CAND
IGFR-G33-4-D5	MC	19.5	2.2	1.0	2.2	HIT
IGFR-G33-4-D6	RPWRVNTSESCL#FVCSLFSGYECWVGG*WAAAGAP	3.4	1.1	1.0	1.1	
IGFR-G33-4-D7	PAMAGMGVQSCHDSFYGWFGCLFSDAEGDRAAAGAP	20.7	15.2	7.0	2.2	HIT
IGFR-G33-4-D8	PAMAGDTSRACPESLNG.FCVVGVALRRWIAAAGAP	20.1	7.0	1.0	7.1	HIT
IGFR-G33-4-D9		14.5	6.6	1.7	3.8	HIT
IGFR-G33-4-D10	PAMARWWRGLCGERWYHRGWVQVQFPWERGAAAGAP	6.4	1.1	1.1	1.0	
IGFR-G33-4-D11	RPWRVPWVLEMPEYGNANLVFYDALQRLAAAGAP	27.7	19.7	1.2	16.5	HIT
IGFR-G33-4-D12	AGHGVCYLAGVFGEALGGGRVSGFAIGQVRAAAGAP	29.8	16.9	3.3	5.0	HIT
IGFR-G33-4-E1	PAMAGRIKEFCSRSFYDQVACLVKGPSWGGAAAGAP	11.0	13.2	2.0	6.5	HIT
IGFR-G33-4-E2	PAMAGRIKEFCSRSFYDQVACLVKGPSWGGAAAGAP	18.9	16.0	3.7	4.3	HIT
IGFR-G33-4-E3	PAMAGISSRSCAENLRFGRAWQGSDVWDCLAAAGAP	22.4	21.3	0.9	22.9	HIT
IGFR-G33-4-E4	PAMASRIPQWCRDSFYEWFECQLLGPRESRAAAGAP	14.5	7.3	1.2	6.1	HIT
IGFR-G33-4-E5	PAMAGAESCYRAKSFYDGLGCLVGEAWWGGAAAGAP	7.8	14.3	1.9	7.4	HIT
IGFR-G33-4-E6	PAMARSGAPRCHDPFYEWFAVEAQEPLRCEAAAGAP	6.0	3.1	1.0	3.1	HIT
IGFR-G33-4-E7	PAMAGMGVQSCHDSFYGWFGCLFSDAEGDRAAAGAP	13.9	13.9	1.9	7.4	HIT
IGFR-G33-4-E8	PAMADISFESCLAQLLGWRAGEGSKRLWRCAAAGAP	11.9	17.1	3.5	4.9	HIT
IGFR-G33-4-E9	PAMANTFLYPCRDPFYHSLADLVGVAMQCGAAAGAP	23.2	24.5	5.2	4.7	HIT
IGFR-G33-4-E10	PAMARRIPRECGDSFYAGLRCLVESPRSDWAAAGAP	9.4	5.8	1.7	3.3	HIT
IGFR-G33-4-E11	PAMASIVCPFCEDSFYNWFAAQVADTRGLWAAAGAP	24.1	33.5	10.1	3.3	HIT
IGFR-G33-4-E12		1.2	0.9	1.0	0.9	
IGFR-G33-4-F1	PAMAWSHSHAYTESYYDWFAAQVLSAGSGRAAAGAP	0.9	1.1	0.9	1.3	
IGFR-G33-4-F2	PAMAGRIKEFCSRSFYDQVACLVKGPSWGGAAAGAP	7.2	8.5	0.9	9.7	HIT
IGFR-G33-4-F3	PAMARSRPPACGDSFYGWFECEVSGLGRRGAAAGAP	2.2	1.4	1.0	1.4	
IGFR-G33-4-F4	PAMAGISYPACEESFYDCLASLVLSPWGSGAAAGAP	12.1	5.2	0.8	6.7	HIT
IGFR-G33-4-F5	PAMAGRIKEFCSRSFYDQVACLVKGPSWGGAAAGAP	16.7	24.2	7.3	3.3	HIT
IGFR-G33-4-F6	PAMAVVAGQYCRDSFYDRLSALVGDAWRCGAAAGAP	13.6	7.4	1.9	3.8	HIT
IGFR-G33-4-F7	PAMACTASRFCAVSFYEWFAAQLPDLGGDSAAAGAP	12.5	16.9	1.2	13.8	HIT
IGFR-G33-4-F8	PAMAGITLQSCGGGFYELLASVVGDTGCRLAAAGAP	20.2	10.9	1.0	11.3	HIT
IGFR-G33-4-F9	PAMAGYICRSCQGSFYGCLAALVRDPRCSRAAAGAP	24.7	33.0	8.8	3.7	HIT
IGFR-G33-4-F10	PAMAGRIKEFCSRSFYDQVACLVKGPSWGGAAAGAP	7.1	10.6	1.2	9.1	HIT
IGFR-G33-4-F11	RPWRVAGAPRCHDPFYEWFAVEAQEPLRCEAAAGAP	1.0	1.0	0.8	1.2	

FIG. 43A-2

Clone #	Sequence	Etag	IGFR	LDH	IGFR/LDH	Binder
IGFR-G33-4-F12	PAMAGMGVQSCHDSFYGWFGCLFSDAEGDRAAAGAP	7.6	4.7	0.6	8.0	HIT
IGFR-G33-4-G1	PAMASICGQSCRDPFYAGLRGLLLEPLQLGAAAGAP	17.6	18.5	1.0	19.5	HIT
IGFR-G33-4-G2	PAMAGVMSKCCSGSFYDWLADLVPEASWSWAAAGAP	6.5	5.7	1.0	5.5	HIT
IGFR-G33-4-G3	PAMASFSGEACGGSFYDCLAGLMRDSSVSRAAAGAP	18.4	7.9	1.1	7.4	HIT
IGFR-G33-4-G4	PAMASFSFYTCMETLLDGFGGQAFNRCRRTAAAGAP	22.5	20.1	1.3	15.6	HIT
IGFR-G33-4-G5	PAMARVIYPTCPRDFYGGLAALVFGPHVCGAAAGAP	22.8	21.7	1.9	11.5	HIT
IGFR-G33-4-G6	PAMAGIGSQACTDPFYYWFEGLVSNGGWCRAAAGAP	5.9	5.3	1.2	4.3	HIT
IGFR-G33-4-G7	PAMAGRIKEFCSRSFYDQVACLVKGPSWGGAAAGAP	18.8	2.1	1.0	2.1	HIT
IGFR-G33-4-G8	PAMAGAESCYRAKSFYDGLGCLVGEAWWGGAAAGAP	23.6	30.3	3.7	8.2	HIT
IGFR-G33-4-G9	PAMADMMSQVCSQSMTGRFSVDFYDGLRCLAAAGAP	17.3	4.6	0.9	5.1	HIT
IGFR-G33-4-G10	PAMARRIPRECGDSFYAGLRCLVESPRSDWAAAGAP	26.8	24.6	5.4	4.6	HIT
IGFR-G33-4-G11	PAMARVIQEACGGSFYDGLACLVYPQGWRGAAAGAP	3.3	1.5	0.9	1.7	CAND
IGFR-G33-4-G12	PAMAGGRSVACQESFYALLGCVVMGPGGGSAAAGAP	24.1	32.1	12.1	2.7	HIT
IGFR-G33-4-H1	PAMAGISFRSCLQALIAGSAGNASEMGCRSAAAGAP	5.9	5.8	1.2	4.8	HIT
IGFR-G33-4-H2	PAMAGIRDSYCQGAFYDWFAGLVDDGLFCQAAAGAP	9.2	4.4	1.0	4.4	HIT
IGFR-G33-4-H3	PAMAGISYQSCEDSFYAWFACTVLDTRGGGAAAGAP	17.8	16.0	1.8	8.9	HIT
IGFR-G33-4-H4	PAMARVIYEACGGSFYDGLACLVYPQGWRGAAAGAP	3.1	3.2	1.1	2.8	HIT
IGFR-G33-4-H5	PAMADMPLLECLDPFYSWFAGQVSDPRFCGAAAGAP	20.1	7.5	0.9	8.0	HIT
IGFR-G33-4-H6	PAMARVIQEACGGSFYDGLACLVYPQGWRGAAAGAP	5.1	2.4	0.8	2.9	HIT
IGFR-G33-4-H7	PAMAGRIKEFCSRSFYDQVACLVKGPSWGGAAAGAP	12.9	11.1	1.1	9.8	HIT
IGFR-G33-4-H8	MC	23.4	23.5	1.6	14.7	HIT
IGFR-G33-4-H9	PAMAHISFHSCLEALQDPEWGQPSAAWRNCAAAGAP	1.2	1.1	0.8	1.3	
IGFR-G33-4-H10	PAMAMTAQESCPDSFYECLAVLVGDRWGGWAAAGAP	7.9	10.4	2.8	3.7	HIT
IGFR-G33-4-H11	PAMAHISFHSCLEALQDPEWGQPSAAWRNCAAAGAP	16.8	23.7	1.3	18.1	HIT
IGFR-G33-4-H12	PAMAGTISQCCEENFYAGLAHLAGVGQWGCAAAGAP	20.4	19.0	4.7	4.0	HIT

FIG. 43A-3

Clone	#	E-Tag	IGFR	IR	Sp/Irr	Binders
B10	DDDKTFYACLEFLLSGNPEGNSGPWDRCR	2.6	29.0	1.0	29.0	HIT
D1	MC	17.0	26.8	1.1	25.1	HIT
A4	DDDKIFYSCLASLLHGGPQRNTGPWERCR	25.2	25.6	1.0	25.6	HIT
A6	DDDKTFYSCLASLLTGPREQNRGAWERCR	22.3	23.3	1.1	21.2	HIT
B1	DDDKSFYSCLASLLTASRLPSRGAWDGCH	18.0	22.8	0.9	25.3	HIT
E4	DDDKSFYSCLGSLLTGAPQPIRGAWDRCR	20.8	20.6	1.1	19.1	HIT
C11	DDDKSFYSCLASLWSGTGGSSRGRWEGCR	22.7	20.4	1.3	16.2	HIT
C2	DDDKTFYSCLGALLAGTGERNLRPWGRCR	19.9	19.1	1.0	19.1	HIT
B6	DDDKTFYSCLGSLLTGPSDPKRGPWGGCR	22.6	19.0	1.2	15.3	HIT
A12	DDDKTFYKCLASVLTGSTQTKRRPWEGCR	13.9	18.8	1.0	18.4	HIT
D2	DDDKPFYSCLATLLTDPPQSQRGAWGRCR	22.5	17.2	1.1	16.1	HIT
C1	DDDKSFYSCLAALLTGSSQSSGGAWMLCR	21.6	16.9	1.1	15.2	HIT
F10	DDDKSFYSCLASLVAGTPWPKGGSWERCR	11.4	16.6	0.9	18.2	HIT
B11	DDDKSFYSCLASLVTGIPRSNSGTQVFCR	7.8	16.1	0.8	20.8	HIT
A9	DDDKSFYSCMASLLTGTPESRRGMGERCG	16.3	15.9	1.3	11.8	HIT
A10	DDDKAFYSCLASLLTGSPPAQGGPWDRCR	8.3	15.6	1.0	16.2	HIT
B9	DDDKFFYSCLASLLSDTPQRRRGPGVRCR	5.9	14.3	0.9	15.4	HIT
A3	DDDKTFYSCLASLLAGNPQPNRAGWEYCR	14.1	13.3	0.9	15.1	HIT
F2	DDDKTFYSCLGSLLLGPPQKNPGPGERCR	9.6	13.3	0.9	14.8	HIT
A7	DDDKMFYSCLESLLIGRWPRNGGSLSRCR	10.9	13.2	1.1	12.5	HIT
G3	DDDKSFYSCLTFLLTGTPQANDASWERCR	9.6	13.2	1.0	13.8	HIT
A11	DDDKSFYSCMAALLSGAPQKSRGRWERCG	4.7	12.7	0.8	15.0	HIT
B7	DDDKAFYRCLAYLLAGRPQASGGGGVRCR	19.7	12.5	0.9	13.5	HIT
D5	DDDKAFYSCLAALRERSPQMSRGTWGGCR	21.8	11.8	1.3	9.3	HIT
E8	DDDKTFYACLAALLGGTAELHDGSLECRG	11.8	11.8	1.3	9.0	HIT
D10	DDDKTFYSCLGSLLTGTLPPARGARNICR	15.1	11.6	1.0	11.6	HIT
D8	DDDKTFYSCLSSLLAGSPLPRRDLWAGCR	11.1	9.2	0.9	10.6	HIT
D12	DDDKAFYSCMASLLAGTPEAQGSAWVRCR	5.4	7.4	0.8	9.0	HIT
A5	DDDKNFYACMESLVSVAPPSSRDPFECRR	16.0	7.3	1.3	5.5	HIT
F6	DDDKSFYSCLASLVSGTA.PNRGPWERCR	4.2	6.9	1.0	7.1	HIT
G5	DDDKIFYSCLASLLDDTAQSRRGQWARCR	4.8	6.4	1.3	5.1	HIT
C6	DDDKIFYSCLGALLSGTPQTSHVTSGRCR	13.6	5.9	1.1	5.4	HIT

FIG. 43B-1

Clone	#	E-Tag	IGFR	IR	Sp/Irr	Binders
D3	TDDKTFYFCLASLLAGTQQQSRGAWERCG	5.1	5.9	0.9	6.5	HIT
A2	DDDKAFYSCLASVLTGSPHPGRSRWERCR	18.6	5.5	0.9	6.0	HIT
B2	DDDKTFYSCLESMLTGTPPPCRGHGERGR	8.0	5.5	1.0	5.6	HIT
D7	DDDKTFESCLEALVSGGSRRERGLWYRCR	10.6	5.5	1.1	5.1	HIT
C12	DDDKAFYSCLSSLLAGTRERHRDTWPRCG	12.0	5.3	1.0	5.3	HIT
B5	DDDKTFHSCLAALVTGTPQQKRGPWERCR	20.0	5.1	1.3	3.9	HIT
E2	DDDKTFYLCLASLQTVTRLGDRVPWERCR	18.0	4.6	0.7	6.4	HIT
F3	DDDKTFYSCLVSLLNGTAKPNRGQWEGCR	3.7	4.5	1.4	3.2	HIT
H2	DDDKSFYSCLASLSNCTPGLLRCQWERCR	7.2	4.4	0.8	5.4	HIT
B4	DDDKTFYSCLSSLLASTPQPNRGAWLCRR	11.9	4.3	1.2	3.6	HIT
G12	DDDKSFYSCLASLSNCTPGLLRCQWERCR	2.2	4.2	1.0	4.4	HIT
F5	MC	8.4	4.0	0.9	4.3	HIT
A1	DDDKTFYSCLGALLSGAPQTYRGPGAGCR	8.1	3.9	1.0	3.9	HIT
E11	DDDKTFYSCLVSLLNGTAKPNRGQWEGCR	4.9	3.8	1.0	3.9	HIT
F9	DDDKHFYSCLSSLLTAPPQSTRGPAGRHC	3.9	3.7	1.0	3.6	HIT
E3	DDDKTFYSCLASLLNGNTQPNGGQWVRCR	1.7	3.6	0.8	4.5	HIT
G4	DDDKVFYTCLASLSTGTPQRQSGEWQRCR	9.4	3.3	1.3	2.5	HIT
E5	DDDKTFYSCLVSLLNGTAKPNRGQWEGCR	4.6	3.2	0.9	3.7	HIT
C10	DDDKPFYSCLASLIQGTPLPERGMWERCR	6.3	3.0	1.0	3.0	HIT
C7	MC	10.6	2.9	1.0	3.0	HIT
H5	DDDKTFYSCVSWLLTGARQRDGVWERCR	5.5	2.9	1.3	2.2	HIT
H3	DDDKTFYSCLVSLLNGTAKPNRGQWEGCR	4.6	2.8	1.1	2.5	HIT
C3	DDDKAFYGCLAALLTGARQPSRGVGERCF	6.0	2.7	1.5	1.8	HIT
F1	DDDKTFYSCLVSLLNGTAKPNRGQWEGCR	1.5	2.6	0.8	3.4	HIT
B3	DDDKTFYSCLASLLAGSPQPKRAGWEYCR	8.6	2.5	1.0	2.5	HIT
D11	DDDKPFYSCLESLVTGRPQADRGVWERCR	4.9	2.4	0.9	2.7	HIT
E10	DDDKTFYSCLTSLSRGSAHGLSGRWERCR	5.0	2.3	0.9	2.7	HIT
F12	DDDDKTFYFCLATLLTGPPVPNREPWACYR	2.5	2.2	0.8	2.7	HIT
D4	DDDKIFYSCLRTLGTNPPEPVRGPFDRCG	3.0	2.1	1.1	1.9	HIT
E6	DDDKTFYSCLVSLLNGTAKPNRGQWEGCR	2.7	2.1	1.4	1.5	HIT
F11	NDDKSFYSCVASLVNEGPSQVGVLGERCR	3.5	2.1	1.2	1.7	HIT
A8	DDDKTFYSCLASMLTGPPHPDRVPWDRCR	8.3	2.0	1.0	1.9	HIT
F7	DDDKKFYSCLVELVNGTSPPARGLWERCR	2.7	2.0	1.0	2.1	HIT
F8	DDDKVFYSCLESLVSGTPEVNGRAWERCR	2.0	1.8	1.0	1.7	CAND
E12	YDDKRFYYCLASLASGTLQTNREQWERCR	1.0	1.7	1.0	1.7	CAND
H1	DDDKTFYSCLESLLNGTPQRNRGQWDPCS	1.9	1.7	0.9	1.9	CAND
H8	DDDKTFYTCLQALITGYERPVGGRWESCR	1.2	1.7	1.2	1.4	CAND

FIG. 43B-2

Clone	#	E-Tag	IGFR	IR	Sp/Irr	Binders
G9	DDDKAFYSCLATLLYGNPPSSRGQWHRCR	1.5	1.6	1.1	1.5	CAND
D6	DDDKVFYSCLESLVSGTPEVNGRAWERCR	1.3	1.5	1.1	1.5	CAND
H6	GDDKTFYACLSSLLYGTADWSQGQRDRCR	3.2	1.5	1.3	1.1	
E7	DDDKSFYSCMESLWTDTPQPNRGRWERCR	3.2	1.4	1.1	1.3	
F4	DDDKTFYSCLASLLTVSPEPSRGPWERCR	1.4	1.4	1.2	1.1	
G6	MC	1.4	1.4	1.0	1.5	
G11	DDDKTFYSCMVQLLTGTPEKSCVTWERWR	1.6	1.4	1.0	1.4	
H4	DDDKTFYSCLVSLLNGTAKPNRGQWEGCR	4.7	1.4	1.0	1.4	
B8	DDDKTFYSCVASLVVGTAQPQCGPWQGWG	9.1	1.3	1.0	1.3	
C8	DDDKTFYSCLAGLVTGPPRQNWGAGDACR	7.7	1.3	1.4	1.0	
E1	DDDKTFYSCMSSLSTTAPQPKSGRWDRCR	2.3	1.3	0.9	1.4	
G10	DDDKTFYSCLASLVNGSLQPNRAPGELCR	1.2	1.3	1.2	1.2	
H10	DDDKTFYSCLVSLLNGTAKPNRGQWEGCR	3.5	1.3	1.0	1.3	
H11	DDDKTFYSCLVSLLNGTAKPNRGQWEGCR	2.6	1.3	1.0	1.3	
D9	DDDKSFYSCLESLLNGGPQQKRGPWEGCR	1.1	1.2	0.9	1.3	
E9	DDDKTFYSCLASLLNGNTQPNGGQWVRCR	1.1	1.2	1.1	1.1	
C9	MC6	1	1.1	1.1	1.0	
G1	DDDKSFYSCLASLSNCTPGLLRCQWERCR	0.9	1.1	0.9	1.3	
H7	DDDKNFYSCLSALLNGNTVSDRGQGERCF	2.2	1.1	0.9	1.1	
H9	DDTFYSCLASLVNGSPQAYGGPREHCR	3.1	1.1	1.1	1.0	
C5	DDDKIFYSCLAWLMTGPAPPYRGPWSCWS	3.3	1.0	1.0	0.9	
G7	DDDKIFYACLSSLSTGTWQPKRGPGERCR	0.9	1.0	0.9	1.1	
H12	DDDKSFYSCLASLSNCTPGLLRCQWERCR	3.7	1.0	1.0	1.1	
C4	DDDKTFYSCLGSLINGTPPPHRGLWQGCR	1.1	0.9	0.9	1.0	
G8	DDDKTFYSCLVSLLNGTAKPNRGQWEGCR	0.7	0.9	1.0	0.9	
G2	DDDKQFYSCLAFLASGIAQPQPGAWELCR	1.1	0.8	1.0	0.8	
B12	DDDKTFYCCLAALLTGAPPPKGGTCERCG	9.3	13.2	0.7	18.3	HIT

FIG. 43B-3

IR

Clone	Sequence	E-Tag	IR	IGFR	IR/IGFR	IGFR/IR
B5	FHENFYDWFARKDSGGSGGSGGSDLCVLEELFWGDSLFDYCTG	17.0	16.9	0.5	35.8	0.0
A3	FHENF.DWFVRQVSGGSGGSGGSNLCVLEELFWGASLFGECSG	13.0	11.8	0.3	35.8	0.0
A8	SHGNFSEWFVRQGYGGSGGSGGSDLCVLEELYWGASLFGYCSG	13.2	13.1	0.4	33.2	0.0
C7	FQESFYDWFVR.VTGGSGGSGGSDLCGVEDLVWGSALSGYCAG	15.1	14.7	0.5	30.6	0.0
B4	FHENFNDWFVREVSGGSGGSGGSDLCVLEELFWGASLFSYCSG	13.2	11.7	0.4	27.6	0.0
B11	SHENFYDWFVR.GPGGSGGSGGSHLCVLEELFWGDSLFGACPG	10.9	9.1	0.3	27.0	0.0
A9	FHENFYDWFARQVSGGSGGSGGSHLCVLEELFWGASLFA.CSD	10.7	12.3	0.5	25.7	0.0
A6	FPDNFYDWFVR.VSGGSGGSGGSHLCVLEELFWGASPFGYCSG	11.6	8.7	0.4	19.8	0.1
A4	**FQENFYDWFGRQI**SGGSGGSGGS**PLCDVEELFWGVSLFGYCTG**	13.6	12.1	2.6	4.6	0.2
C8	FQENFYDWFVR.ASGGSGGSGGSHLCALEEQFWGSSQFRYCSG	16.0	14.5	3.2	4.5	0.2
A10	FHENFYDWFARQVYGGSGGSGGSHLCVLEELF.GASLFATCSD	10.6	6.0	1.5	3.9	0.3
D11	FHENFYDRIVRQVAGGSGGSGGSALCVREELF.GDSLFGDCSG	12.4	5.5	1.5	3.6	0.3
D4	FHKNFYDWFDRQVSGGSGGSGSSRLCDLEELFWGASL.GHCSG	15.4	9.8	3.9	2.5	0.4
C1	FHENFYDWFIRQDSGGSGGSGGSHLCAFEELLGGASPFGYCSG	16.8	2.7	1.3	2.1	0.5
D12		11.7	8.7	4.6	1.9	0.5
D8	SNENFYDWFDR.VSGGSGGSGGSHLCLLEELSWGASLFGYCYG	15.8	9.6	7.4	1.3	0.8
C11	FHESFYDWFDRQVSGGSGGSGGSHLCVLEE.ELGASVFGCCSG	11.0	5.8	5.4	1.1	0.9
C4	FHETFYDWFDR.VSGGSGGSGEELFGGASLFGYPSG	16.7	13.2	15.0	0.9	1.1
D1	SHENFYDWFGRQVSGGSGGSGGSNLCDLDEVS.GASLCGYRSG	16.2	5.5	7.1	0.8	1.3
C6	FH.NFYDWFFCQVPEWIPMTLAVLTCAVLEEPIWGDSLFGYG.E	16.1	1.7	2.2	0.8	1.3
A5	SHENFYDWFVRQV.GGSGGSGGSHLCDLEELLGGASLMGSCSG	16.0	8.7	12.9	0.7	1.5
B8	SHENFYDWFVR.VSGGAAAGAPPAMASHENFYDWFVR.VSGG	15.2	8.9	13.9	0.6	1.6
D2	FHENFYDWFIR.VGGGSGGSGGSDLCVLEDDCSRAAAGAP	13.9	8.4	13.1	0.6	1.6
A2	DYKDASVSGTFHDAFYEWFWR.VGS	13.4	6.9	12.6	0.6	1.8
C12	FHENFYDLVPSAGSWWIRWLWRF.PVRLGRTVLGCFSDR.LFW	9.2	4.4	6.8	0.6	1.5
B9	FHENFYDWFDRQVSGGSGGSVRAAAGAP	17.8	8.2	16.3	0.5	2.0
B1	VHENFYDWFDRQVSGGSGGSGGSQLCDL.EVIWGASLFGYCTG	18.1	7.3	13.4	0.5	1.8

FIG. 44A-1

Clone	Sequence	E-Tag	IR	IGFR	IR/IGFR	IGFR/IR
B2	FHENFYDWFDR.VSGGSGGSGGSHLCVPEEQFWGASRFGYCSA	16.2	7.0	13.6	0.5	1.9
D9	FHDNFYDWFVRQVSGGSGGSGGSRQCQGASL.GYCSG	17.0	6.6	13.8	0.5	2.1
D5	OUT OF FRAME	16.1	4.8	10.5	0.5	2.2
C9	FHEDFYDWFVR.VPGGSGGSGGSHLCVPGYCSS	17.2	5.1	14.2	0.4	2.8
B7	FRENFYDWFVC.VSGGSGGSGGSNLCVLEEAAAGAP	15.9	4.0	10.6	0.4	2.7
D6	OUT OF FRAME	15.6	4.2	12.8	0.3	3.0
C5	GHDNFYDWFVRQVSGGSGGSGGSHLCV.GAFPWGYCSD	15.2	3.6	10.7	0.3	3.0
D3	FH.NFYDWFVRQVYGGSGGSGTGAAAGAP	16.2	3.5	12.0	0.3	3.4
C10	BAD SEQUENCE	11.2	2.5	7.6	0.3	3.1
A7	FHENFYDWFGRQVYGGSGGSGGSPVCILGELS.GGALFGDCSG	15.5	1.8	5.1	0.3	2.9
A12	FHENFYDWFVR.LSGGSGGSGGSHLCVPEERLWGDPLFGYCSG	8.7	1.2	3.5	0.3	3.0
D7	FH.NFYDWFVRQVSGGSGGSGGSHPAR	16.2	3.0	11.9	0.2	4.0
A11	FHENFYDWFVRQVTGGSGGSGGSHLCVLEELS.GAALPGYCSG	11.8	1.0	4.0	0.2	4.1
D10	VQGSFYDWFVRQVSGGSGGSGGSHLC.GSG	12.7	1.0	6.3	0.2	6.6
C3	FHENFYEWFVRQVSGGSGGSGGSHRCDVEELH.CASG	16.8	0.6	2.5	0.2	4.2
A1	DYKDGGYWGSFYEGLM.LVQSGTSG	13.6	1.7	12.5	0.1	7.1
B6	OUT OF FRAME	12.7	1.0	8.1	0.1	8.1

Non-Binders:

B12	FHENFYDWFDRQVSGGSGGSGGSHRCVLEERFWGASLFG.CSG	7.8	0.5	1.6	0.3	3.3
B10	SHENFYDWFVHQVSGGSGGSGGSHLCVLEERF.GPSLFGYCSG	10.8	0.6	1.4	0.4	2.3
B3	FHANFYDWFFRQVSGGSGGSGGSDLCVLQDMF.GGSGAAAGAP	16.9	0.7	1.2	0.6	1.7
C2	FQDNFYDWFVRQISGGSGGSGGSHLCVLESWF.GASLFGYCSG	14.8	0.5	0.8	0.6	1.7

FIG. 44A-2

IGFR

Clone	Sequence	E-Tag	IR	IGFR	IR/IGFR	IGFR/IR
H11	FHETFYD.LGRLVFGGSGGSGGSHLCVPEELFWGTSLLSYCSG	9.3	0.4	4.0	0.1	11.4
F11	FHENFYDWFVRQVSGGSGGSGGSHL.GSG	12.5	0.8	5.2	0.2	6.5
E2	FHENFYDWFVRQVSGGSGGSGGSHRCGLEEPV.GASLVGYCAG	13.4	1.3	7.5	0.2	5.7
G7	FHANFYDWFVRQV.GGSGGSGGSG	16.1	2.1	9.8	0.2	4.7
G12	FHEDFYDWFVRQVGGGSGGSGGSHLCVREELF.GASLLGDCSG	9.4	1.2	5.5	0.2	4.6
H7	.HENFYDWFVRQLSGGSGGSDGSHLFGYGSG	7.2	0.6	2.7	0.2	4.5
G11	OUT OF FRAME	11.4	1.4	5.8	0.2	4.3
F7	FHENFYDWFDRQVGGGSGGSGGFSPVRTGRTVLGGFSVRLLLW	15.0	2.7	10.9	0.2	4.1
G1	SHDNFYDWFVR.VSGGSGGSGGSPLCVLGNCSG	11.3	2.8	10.6	0.3	3.8
E8	FYDNFYHWFDR.VSGGSGGSGGSHLCVLEERVCGASLFDYRSG	13.5	0.9	3.4	0.3	3.7
E9	FSEHFYDWFARQVSGGSGGSGGSHLCVLDERF.GASMVGYCSG	14.5	0.7	2.3	0.3	3.6
G2	FPENFYDWFDRQVSGGSGGGASLFG.GSG	15.3	3.8	13.1	0.3	3.5
E3	FHENFYDWFDRQVSGGSSGSGGSHQCVQEERFWGASLCGYCSG	15.9	1.9	6.7	0.3	3.5
E12	FHDSFYDWFVRQVSGGSGGSGGSHLCGLEELF.GASRFGDCSG	10.0	2.3	6.8	0.3	2.9
E5	OUT OF FRAME	14.7	3.7	9.6	0.4	2.6
F8	FHGDFYDWFVR.VSGGSGGSGGSHLCVLEELYCSG	13.7	3.6	9.5	0.4	2.6
E6	FHDNFYDWFVR.VSGGSGGSGGSHLCVVEERFWGGSPIGYCSG	13.3	3.0	7.3	0.4	2.5
G8	OUT OF FRAME	13.9	4.5	10.5	0.4	2.4
E1	FQDNFYDWFVRQVSGGSGGSGGSHRCVLEGCSG	13.4	5.8	13.3	0.4	2.3
H12	FHENFYDWFDRQVSGGSACLFGYCSG	9.8	3.9	8.5	0.5	2.2
F2	YHENFYDWFVR.VSGGSG	14.4	6.2	12.8	0.5	2.1
H6	VHESFYDWFVR.VAGGSGGSGGSHLCDVDCSG	11.5	4.8	9.6	0.5	2.0
H4	FHDNFYDWFDRQVSGGSGGSGSPFG.RSD	11.2	5.3	10.0	0.5	1.9
H5	FH.HFYDWFDRQVSGGSGGSGGSLLCVGEEPFWGASLFAYCSG	11.8	4.4	8.5	0.5	1.9
E7	FHENFYDWFVRQVSGGSGGSGG	15.4	7.8	14.0	0.6	1.8
F5	FHESFYDWFVR.VPGGSGGSGGSQLCVQEELFEGDSLLGDCSG	16.8	7.3	12.9	0.6	1.8
F10		13.9	5.9	10.8	0.5	1.8
E10	FHENFYEWFDRQVSGGSGGVLDERF.GACPSGYCSG	10.6	5.1	8.9	0.6	1.8

FIG. 44B-1

Clone	Sequence	E-Tag	IR	IGFR	IR/IGFR	IGFR/IR
F4	FHDNFYDWFVR.VAGGSGGSGGSHLCVPEELFWGASLFGYCSG	15.7	3.1	5.6	0.6	1.8
H2	FGEDFYDWFVR.VSGGSGGSGGSHLCVLDELFWDASPFGFCPG	11.4	2.5	4.6	0.6	1.8
E11	.HDNFYGWFDRQVSGGSGGSGGSHLCVLDELLWGASLFGYCS	11.7	1.3	2.2	0.6	1.7
F12	FQENFYDWFVR.VSGDELSGGASQCGSCSG	10.6	7.0	9.6	0.7	1.4
F9	SHESFYDWFVRQVSGGSGGSGGSDLCVWEELCGGAPLVG.GSS	16.0	9.9	13.3	0.7	1.3
E4	FPENFYDWFDRQVSGGSGGSGGSSG	16.4	13.4	15.8	0.8	1.2
H10	FRENFYDWFERQVSGGSGGSGGSHLCVLEELSWGASTFGSCSG	10.8	7.8	9.1	0.9	1.2
F3	IHVDFYDWFAR.VSGGSGGSGGSSLCVLDELFWDASLFGDCAG	14.2	3.9	4.6	0.8	1.2
G6	FHASFYDWFDRQVSGGSGGSGGSHLCDLEGLFWGAAPFGYCSG	16.2	11.0	12.1	0.9	1.1
H3	SDANFYDWFLR.VSGGSGGSGGSHLCALEEQFWDASLFGDCSG	13.1	9.8	11.1	0.9	1.1
G5	FHDKFYDWFVS.VAGGSGGSGGSHLCVLEDRFWGSSLSGYCSG	14.7	7.1	7.9	0.9	1.1
H9	FHDNFYDWFVRQVTDGSGGSGGSQLCVVEDLFWDASRFGYC.G	13.1	8.2	8.0	1.0	1.0
G3	VSEDFYEWFVR.ASGGSGGSGGSNLCVLEELFWGSSLIGDCSG	13.7	11.7	2.5	4.6	0.2
G4	FPENFYDWFVRQVSGGSGGSGGSHLCVLEEL.WGASMFGYCSG	10.0	4.3	0.7	6.0	0.2
F6	FQENFYDWFVRQVSGGSGGSGGSHLCVLEALFWGASLFG.CSG	5.6	9.0	0.4	21.2	0.0

Non-Binders:

H1	DYKDGRGGRRF.GRSSVVLWKRL.R	1.2	0.7	0.5	1.5	0.7
G10	DTKTFIGITGVLPRLSAV.GFWGGSW	1.7	0.3	0.3	0.8	1.2
G9	CHENFYVWFVSQVAGGSGGSGGSRLCIM.ELFRGASLFGYSSG	2.0	0.4	0.5	0.9	1.1
F1	FHANFYDWFVR.VSGGSGGSGGSHLCVLEELVSGPSLLGYCSG	14.5	0.6	1.5	0.4	2.3
H8	FHEKFYDWFDL.LSGGSGGSGGSHLCVREEPFWGASLFGYCPG	9.7	0.6	1.5	0.4	2.3

FIG. 44B-2

IGFR Binders with change in Cys

Clone	Sequence	E-Tag	IR	IGFR	IR/IGFR	IGFR/IR
F8(X14)	HLCVLEELFWGASLFGYCSG					
D8(X6)	WLDQEWAWVQCEVYGRGCPS					
G1(X4)	SHDN**FYDWF**VR.VSGGSGGSGGSPLCVLGNCSG	11.3	2.8	10.6	0.3	3.8
E8(X6)	FYDN**FYHWF**DR.VSGGSGGSGGSHLCVLEERVCGASLFDYRSG	13.5	0.9	3.4	0.3	3.7
E3(X10)	FHEN**FYDWF**DRQVSGGSSGSGGSHQCVQEERFWGASLCGYCSG	15.9	1.9	6.7	0.3	3.5
F8(X6)	FHGD**FYDWF**VR.VSGGSGGSGGSHLCVLEELYCSG	13.7	3.6	9.5	0.4	2.6
E1(X4)	FQDN**FYDWF**VRQVSGGSGGSGGSHRCVLEGCSG	13.4	5.8	13.3	0.4	2.3
H12(X4)	FHEN**FYDWF**DRQVSGGSACLFGYCSG	9.8	3.9	8.5	0.5	2.2
H6(X3)	VHES**FYDWF**VR.VAGGSGGSGGSHLCDVDCSG	11.5	4.8	9.6	0.5	2.0
E10(X4)	FHEN**FYEWF**DRQVSGGSGGVLDERF.GACPSGYCSG	10.6	5.1	8.9	0.6	1.8
F12(X2)	FQEN**FYDWF**VR.VSGDELSGGASQCGSCSG	10.6	7.0	9.6	0.7	1.4
F9(X5)	SHES**FYDWF**VRQVSGGSGGSGGSDLCVWEELCGGAPLVG.GSS	16.0	9.9	13.3	0.7	1.3

IGFR Binders with loss of F8

G7	FHAN**FYDWF**VRQV.GGSGGSGGSG	16.1	2.1	9.8	0.2	4.7
F2	YHEN**FYDWF**VR.VSGGSG	14.4	6.2	12.8	0.5	2.1
E7	FHEN**FYDWF**VRQVSGGSGGSGG	15.4	7.8	14.0	0.6	1.8
E4	FPENFYDWFDRQVSGGSGGSGGSSG	16.4	13.4	15.8	0.8	1.2

IGFR Binders with loss of Cys in F8

F11	FHEN**FYDWF**VRQVSGGSGGSGGSHL.GSG	12.5	0.8	5.2	0.2	6.5
H7	.HEN**FYDWF**VRQLSGGSGGSDGSHLFGYGSG	7.2	0.6	2.7	0.2	4.5
F7	FHEN**FYDWF**DRQVGGGSGGSGGFSPVRTGRTVLGGFSVRLLLW	15.0	2.7	10.9	0.2	4.1
G2	FPEN**FYDWF**DRQVSGGSGGGASLFG.GSG	15.3	3.8	13.1	0.3	3.5
H4	FHDN**FYDWF**DRQVSGGSGGSGSPFG.RSD	11.2	5.3	10.0	0.5	1.9

FIG. 44B-3

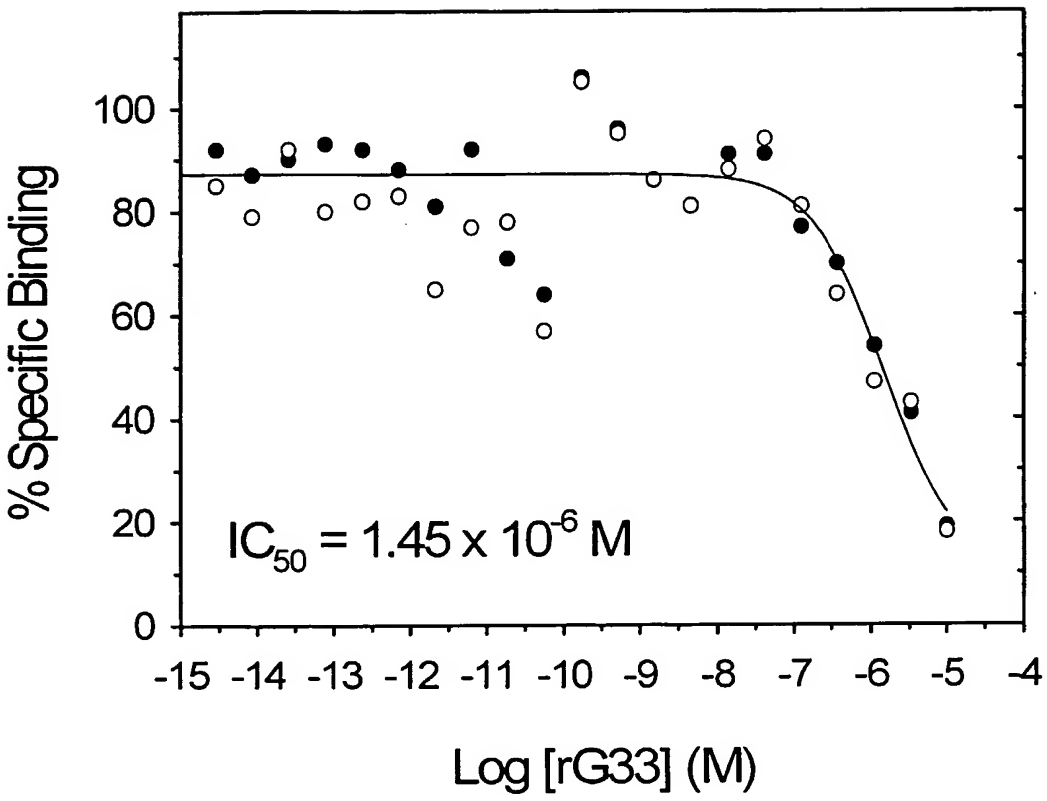

% Specific Binding
100
80
60
40
20
0
-15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
Log [rG33] (M)
IC50 = 1.45 x 10-6 M

FIG. 45

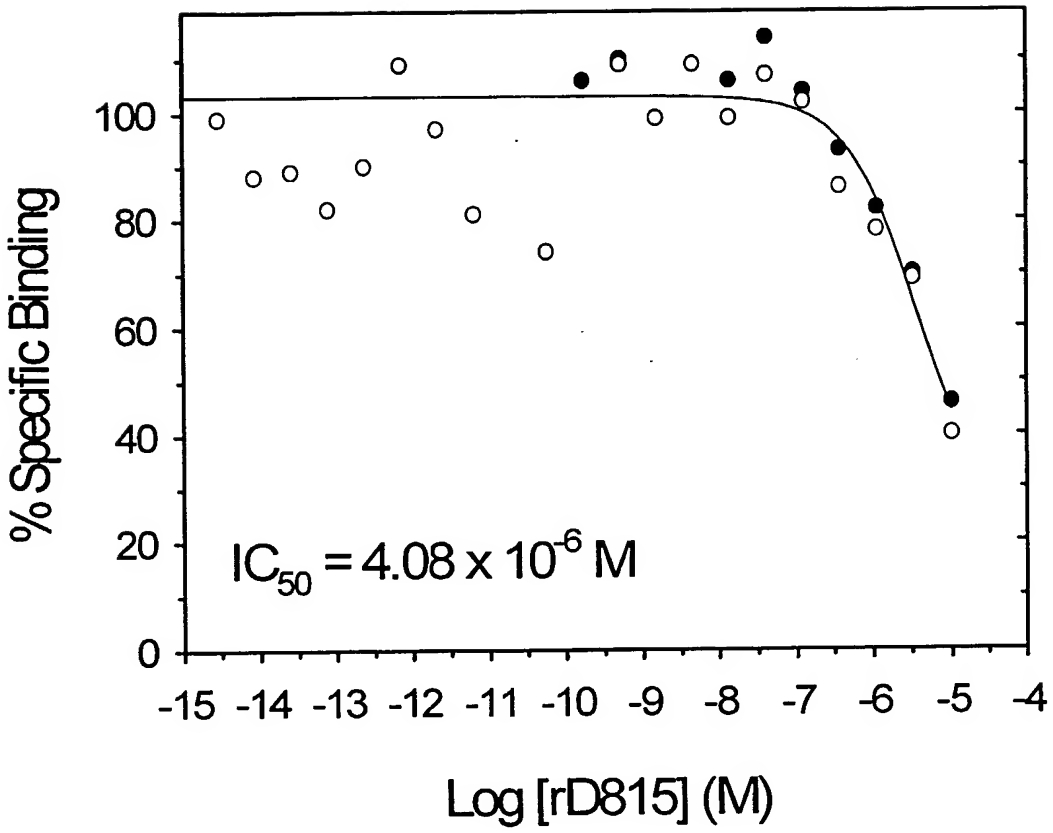

% Specific Binding
100
80
60
40
20
0
$IC_{50} = 4.08 \times 10^{-6}$ M
-15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
Log [rD815] (M)

FIG. 46

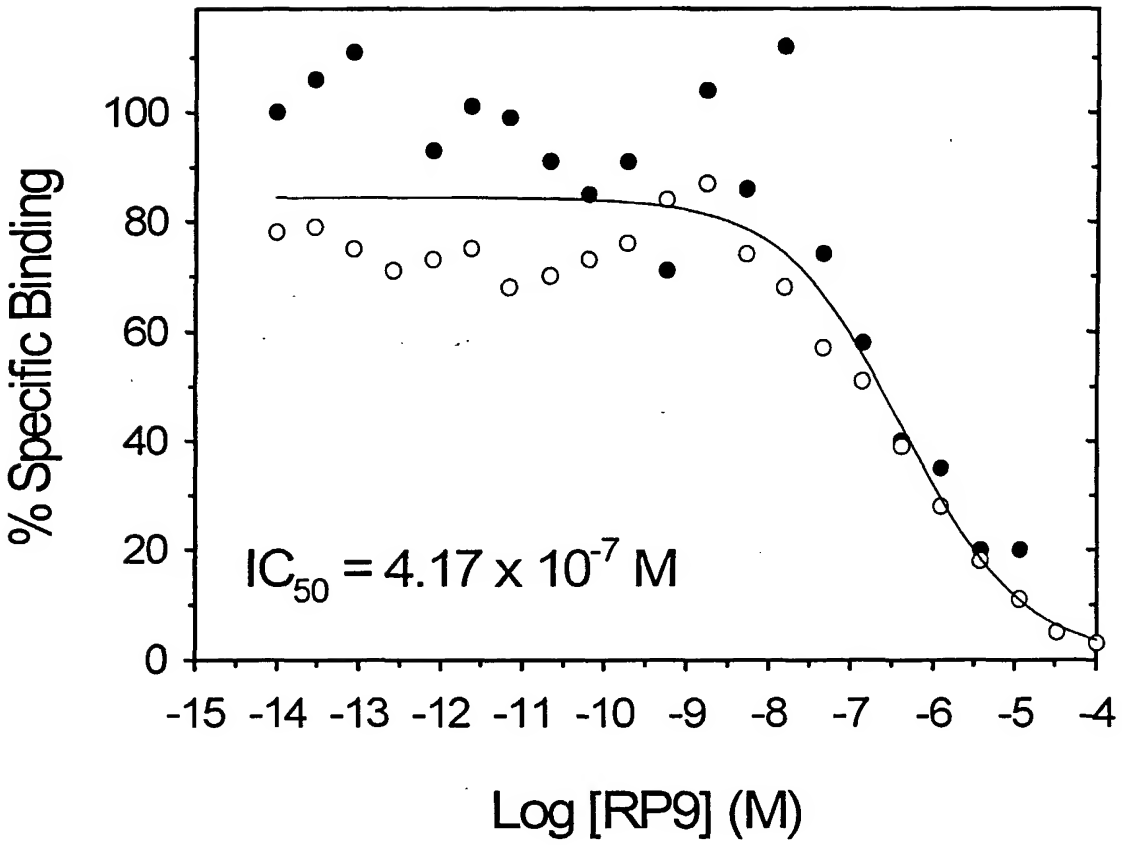

% Specific Binding
100
80
60
40
20
0
-15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
Log [RP9] (M)
IC50 = 4.17 x 10-7 M

FIG. 47

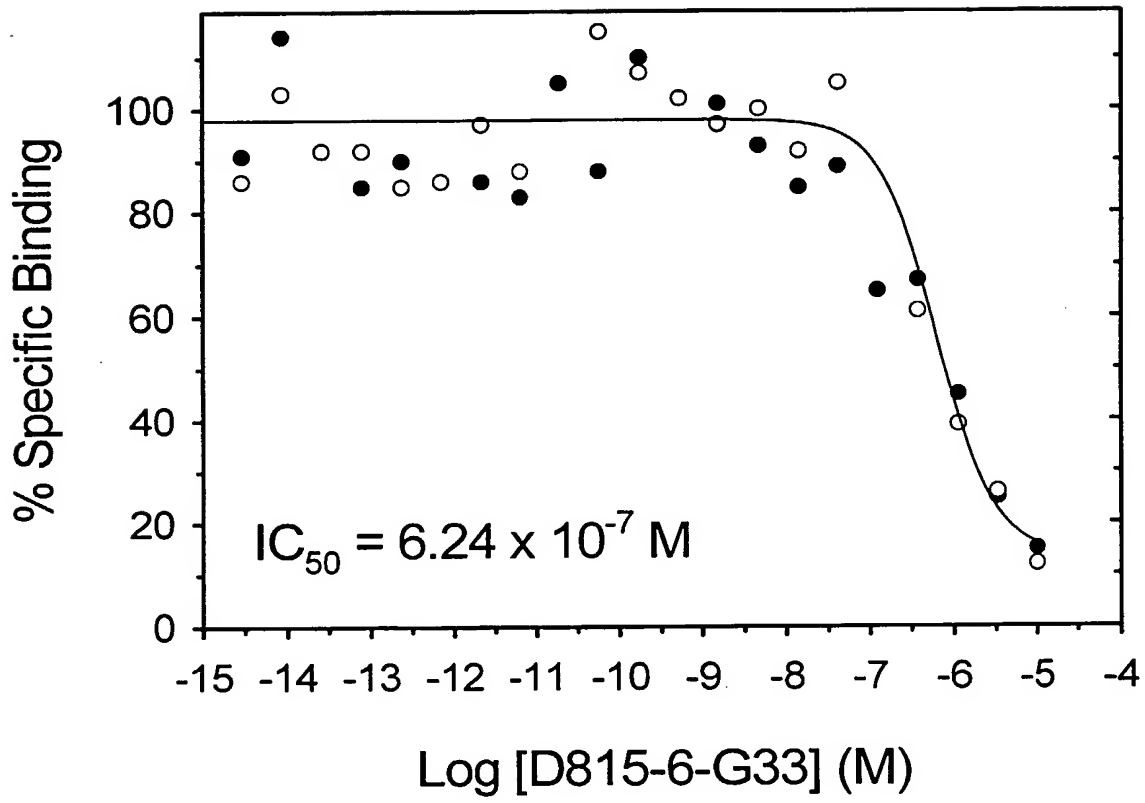

% Specific Binding
100
80
60
40
20
0
-15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
IC50 = 6.24 x 10-7 M
Log [D815-6-G33] (M)

FIG. 48

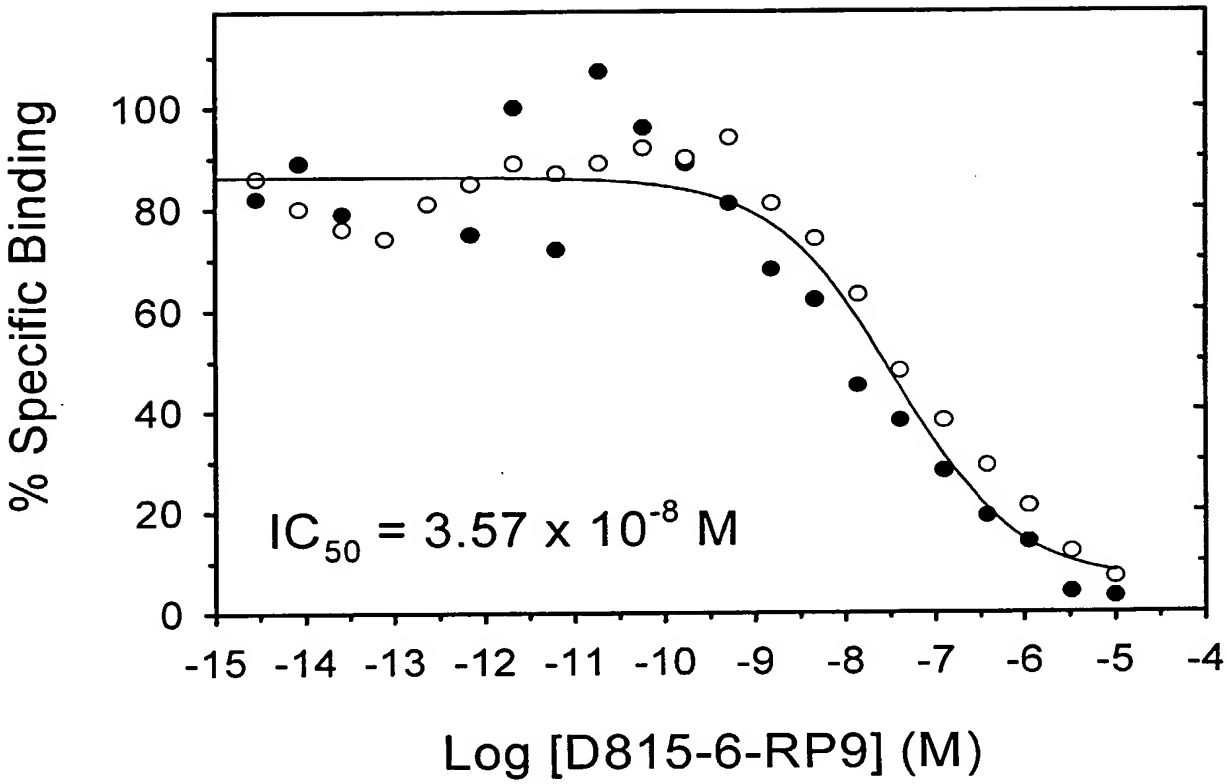

% Specific Binding
100
80
60
40
20
0
-15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
IC50 = 3.57 x 10-8 M
Log [D815-6-RP9] (M)

FIG. 49

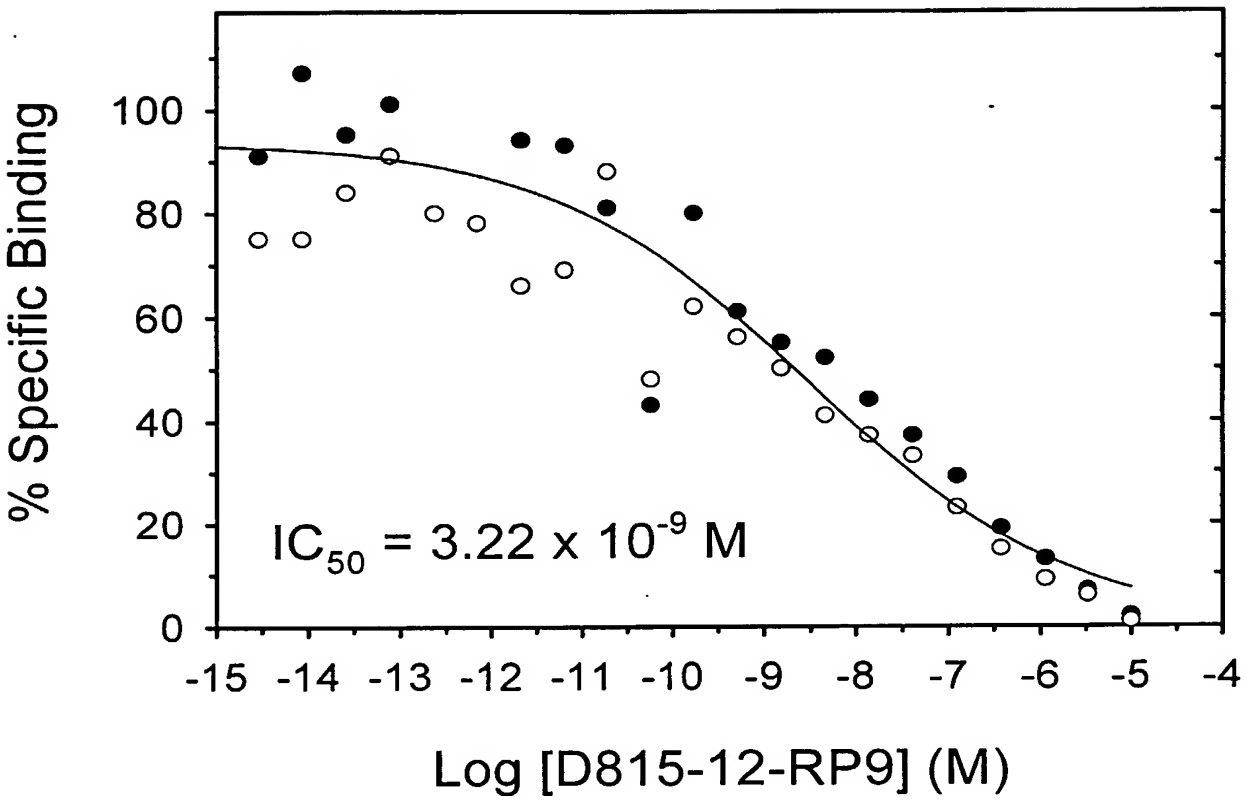

% Specific Binding
100
80
60
40
20
0
-15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
IC50 = 3.22 x 10-9 M
Log [D815-12-RP9] (M)

FIG. 50

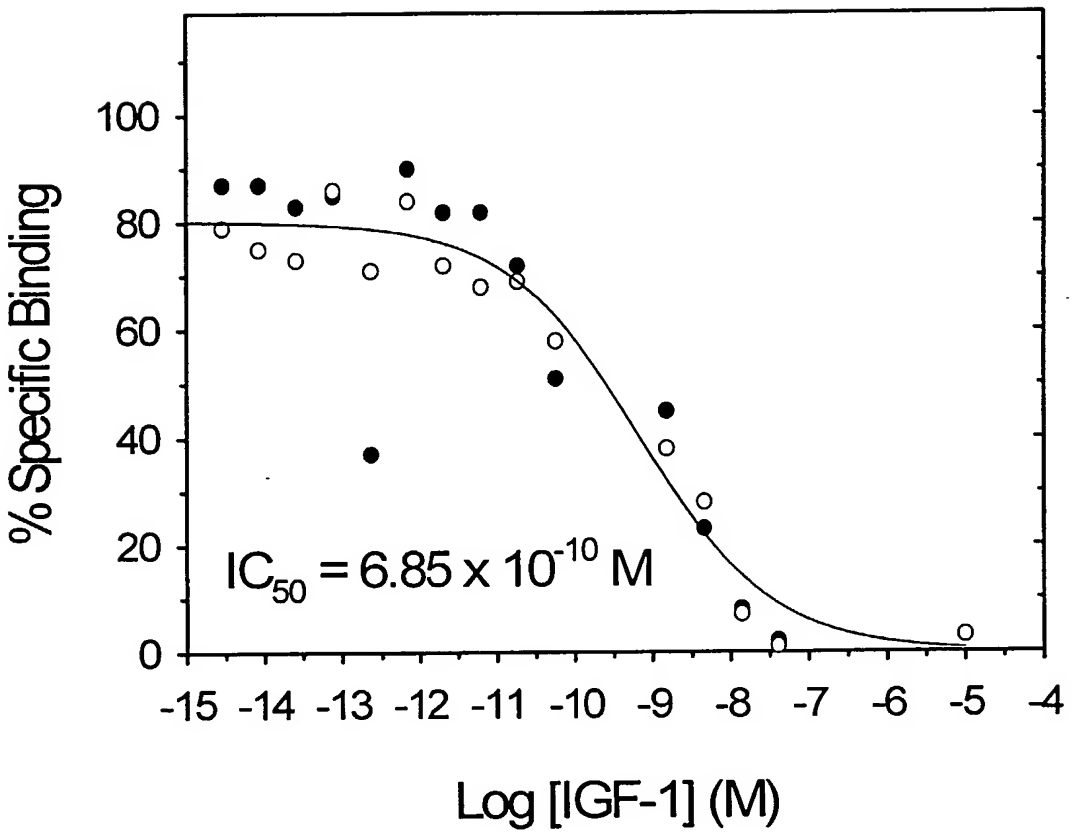

% Specific Binding
100
80
60
40
20
0
$IC_{50} = 6.85 \times 10^{-10}$ M
-15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
Log [IGF-1] (M)

FIG. 51

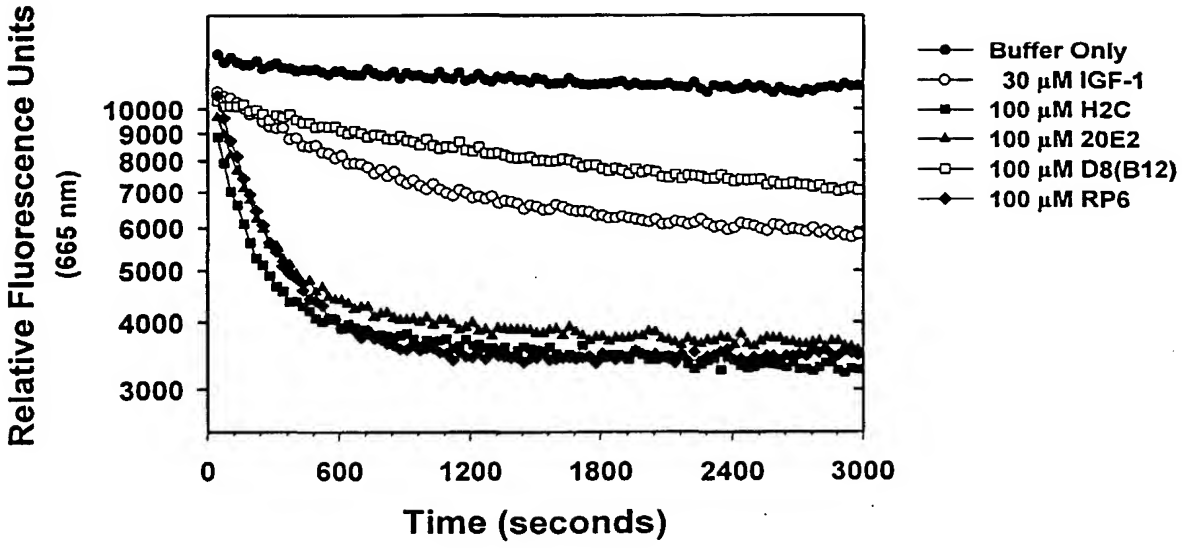

Relative Fluorescence Units
(665 nm)
10000
9000
8000
7000
6000
5000
4000
3000
0
600
1200
1800
2400
3000
Time (seconds)
Buffer Only
30 µM IGF-1
100 µM H2C
100 µM 20E2
100 µM D8(B12)
100 µM RP6

FIG. 52

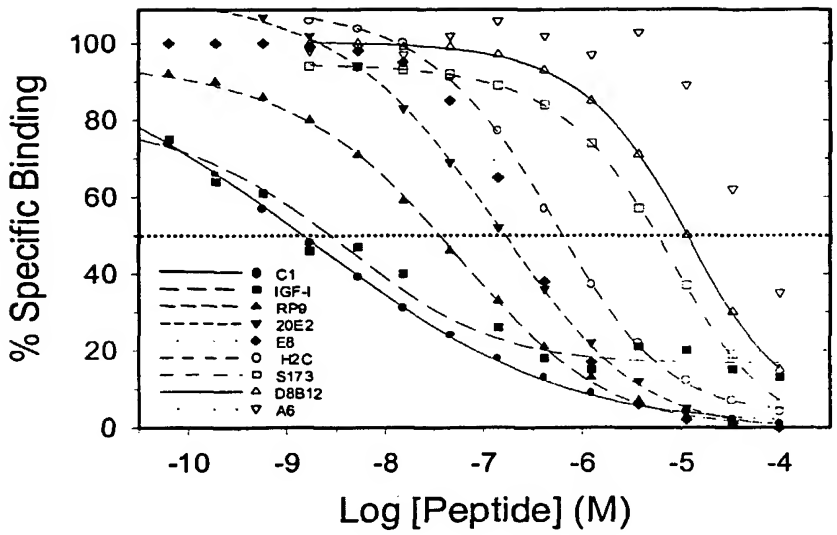

% Specific Binding
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
-4
Log [Peptide] (M)
C1
IGF-I
RP9
20E2
E8
H2C
S173
D8B12
A6

FIG. 53

RP6 vs IGFR

Clone #	Sequence	Etag	IGFR	IR	IGFR/IR	IR/IGFR	SEQ ID NO:
Design	DYKDDDDKTFYSCLASLLTGTPQPNRGPWERCR (FLAG)						
RP6-IGFR-F9	TFYSCLAALLTDRPRPHGGPGVRCR	13.4	9.8	1.1	9.1	0.1	
RP6-IGFR-E9	SFYSCMASLLTGTPQPSCGPRGWFG	14.4	9.8	2.1	4.7	0.2	
RP6-IGFR-H12	SFYSCLASLLTGTPEPDCVRSPQRL	7.8	9.8	3.7	2.7	0.4	
RP6-IGFR-C11	TFYSCLSALVTGTRLPNRGPWESCR	12.8	9.6	1.1	8.4	0.1	
RP6-IGFR-G4	TFYSCLACLVTGTAEKCGCTWERSR	12.7	9.1	3.1	2.9	0.3	
RP6-IGFR-E7	AFYACMASLLSGSAMPGCGAGARSR	14.1	8.7	2.1	4.2	0.2	
RP6-IGFR-G8	TFYSCLASLVTGTPLRNGGVGDRCR	13.2	8.6	1.5	5.9	0.2	
RP6-IGFR-B8	TFYSCLASLVACTPQPKGGACGGCH	11.5	8.3	1.8	4.6	0.2	
RP6-IGFR-D11	AFYSCLATLASGTRLCNRGLWDSGR	15.7	7.8	2.7	2.9	0.3	
RP6-IGFR-D8	TFYSCLDSLVQ.PAASDCAVGTFR	12.1	7.5	1.7	4.6	0.2	
RP6-IGFR-$F8_4$	IFYLCLASLLNGTTQPNCGP.ELSR	11.7	7.5	1.9	4.0	0.3	
RP6-IGFR-B6	RFYACLAALLNGTPRLSGGPGILCR	13.4	6.9	1.0	6.6	0.2	
RP6-IGFR-E4	NFYGCLAALLSGTPHSQRGAWEGCR	9.4	6.8	1.0	7.0	0.1	
RP6-IGFR-C6	YFYSCLASLVTDSELSKRGPCEGPR	13.4	6.0	1.2	5.0	0.2	
RP6-IGFR-B9	TFYSCMAAL.NGTREPDRCSRGGSR	10.6	5.9	2.2	2.7	0.4	
RP6-IGFR-H6	NFYSCLA.LVTGPAQPNSTQWPVCR	9.4	5.7	1.3	4.4	0.2	
RP6-IGFR-$F11_6$	TFYNCLASLLNGTPESNGVQ.DFCR	13.3	4.1	3.4	1.2	0.8	
RP6-IGFR-A7	TFYSCLASLVSGGPQPNRGRWEHCR	7.2	4.0	1.0	4.0	0.3	
RP6-IGFR-H5	TFYSCLGSLLTGTPKPEGGPWESCR	6.9	2.9	0.9	3.4	0.3	
RP6-IGFR-A12	IFYSCLASLLTGSAQPNRCVGERGR	3.1	2.7	1.0	2.6	0.4	
RP6-IGFR-G6	TFYSCMASLLASTPHPTRGRW.GCG	14.2	15.3	1.6	9.7	0.1	
RP6-IGFR-G3	HFYSCLAQLLAGTPKSTRGPGERCR	13.8	15.0	1.0	15.0	0.1	
RP6-IGFR-B12	RFYSCLASLMRGTPLASRGQRVICR	12.7	14.9	1.0	14.6	0.1	
RP6-IGFR-C5	TFYSCLADLLASSSERYRGPRDRCR	14.2	14.9	1.1	13.2	0.1	
RP6-IGFR-F4	TFYSCLASLLTGTPQRNGGSGDRCR	11.8	14.6	2.8	5.2	0.2	
RP6-IGFR-G7	TFYSCLAFLVVGTAEQKRGSWERCR	14.3	14.5	1.3	10.9	0.1	
RP6-IGFR-C10	AFYSCLASLLSGAAQCNRGE.ERSR	16.5	14.0	6.6	2.1	0.5	
RP6-IGFR-E5	TFYSCLASLLSGPPQGRGGLRKRCS	14.5	13.8	1.8	7.8	0.1	
RP6-IGFR-D5	NFYTCLSALLAGPPLPNRCTGERGR	14.5	13.8	3.9	3.5	0.3	
RP6-IGFR-E12	PFYSCLAALATGAPESNRGGWGRCR	12.9	13.6	3.4	4.0	0.3	
RP6-IGFR-B11	NFYSCLSALLSGAPQPHGSSWERCR	14.7	13.4	1.1	12.4	0.1	
RP6-IGFR-G10	TFYSCLAALVTGTPLPHCGTGERNP	11.9	13.4	4.0	3.4	0.3	
RP6-IGFR-E10	TFYSCLSALLIGTPQPNRGPGDGCR	14.1	12.8	1.0	13.4	0.1	
RP6-IGFR-D6	TFYSCLASLLTGPPHQKHGPWGRCR	12.3	12.7	0.9	13.5	0.1	
RP6-IGFR-F6	TFYSCMASLMTGPPPPNRGKWGCWR	13.1	12.5	2.0	6.3	0.2	
RP6-IGFR-F5	TFYSCLASLVMGTPLTNRCQWERGR	12.5	12.3	5.7	2.2	0.5	
RP6-IGFR-C9	SFYSCLA.LVTGSRGTKRGPSERCH	11.2	12.0	1.1	10.8	0.1	
RP6-IGFR-B3	TFYSCLGSLLAG.SPSRGCLWEGGR	11.7	10.8	6.5	1.7	0.6	

FIG. 54A

Clone #	Sequence	Etag	IGFR	IR	IGFR/IR	IR/IGFR	SEQ ID NO:
RP6-IGFR-D7	EFYSCMAALLRVSLKQSSASWGRCR	14.6	10.3	1.8	5.6	0.2	
RP6-IGFR-H7	TFYSCLA.LVTGSPQPNRCPWERGR	9.8	10.1	1.4	7.1	0.1	
RP6-IGFR-F10	VFYSCMASLLAGVPLTNCGPGER.R	10.9	10.1	4.6	2.2	0.5	
RP6-IGFR-C3	TFYECLASLLADTPQPNPGPWERCR	10.8	1.3	0.9	1.4	0.7	
RP6-IGFR-B10	IFEYCLAPRLTGSPQPYRYPWERGR	10.6	1.3	1.3	1.0	1.0	
RP6-IGFR-C12	TFYSCLD.LLTGGPQPNRGAGDHCR	1.1	1.2	0.2	6.2	0.2	
RP6-IGFR-A5	TFSSCIPPLMSDTSHPSRGQCVRCR	2.3	1.1	1.0	1.0	1.0	

FIG. 54B

RP9NPB25 VS IGFR

Clone #	Sequence	E-Tag	IGFR	IR	IGFR/IR	IR/IGFR	SEQ ID NO:
Design	**GSLDESFYDWFERQLGXXXXXXXXXXXXXXXXXXXXXXXXX**	--	--	--	--	--	
RP9NPB25-IGFR-F11	GSLDESFYDWFERQLGAVVGRQDGGPITRDVGGDGRRGYNV	10.9	15.0	11.6	1.3	0.8	
RP9NPB25-IGFR-E7	GSLDESFYDWFERQLGADTKSPRWGSGTREHQGARGGAPGR	12.6	14.9	13.7	1.1	0.9	
RP9NPB25-IGFR-E6	GSLDESFYNWFERQLGSYQDSDRNPGLRSALDRAGDSPLLS	13.0	14.5	13.2	1.1	0.9	
RP9NPB25-IGFR-A8	GSLDESFYDWFERQLGSDSRVESAVGMPGRGGRRNLGEGIR	11.8	14.2	10.9	1.3	0.8	
RP9NPB25-IGFR-G12	GSLDESFYDWFERQLGHGVDETREAELPQISGQQANGVPNQ	10.8	14.2	13.0	1.1	0.9	
RP9NPB25-IGFR-C11	GSLDESFYDWFERQLGRGAVDEGQRSSAVWGAVGRRDDDQR	12.4	14.2	12.5	1.1	0.9	
RP9NPB25-IGFR-C10	GSLDESFYDWFERQLGLVMGEGRAETGRDHRCLSVGSACLE	12.8	14.1	10.3	1.4	0.7	
RP9NPB25-IGFR-F8	GSLDESFYDWFERQLGVAPGRVSAVVGRGHGDASGGGRRTG	12.6	14.0	9.6	1.5	0.7	
RP9NPB25-IGFR-B9	GSLDESFYDWFERQLGLSADQSQTPAVRGAPGRRGL	11.5	13.8	8.8	1.6	0.6	
RP9NPB25-IGFR-E4	GSLDESFYNWFERQLGNGGNDSGELRESRASRGGSIHKNGA	11.4	13.5	12.5	1.1	0.9	
RP9NPB25-IGFR-C6	GSLDESFYDWFERQLGIKGARVVLGQEVCELESRSEGGSCI	15.0	13.4	7.8	1.7	0.6	
RP9NPB25-IGFR-E11	GSLDESFYDWFERQLGTLAQPVRSAPTRAAVGGSHGEQKVG	11.5	13.4	11.7	1.1	0.9	
RP9NPB25-IGFR-G11	GSLDESFYDWFERQLGVKGGQGGVSVGVGQPGTAIRRNLGG	9.8	13.3	3.6	3.7	0.3	
RP9NPB25-IGFR-B12	GSLDESFYDWFERQLGQHQSLGRGGGRGASFSSDGLGNKGG	10.6	13.3	10.1	1.3	0.8	
RP9NPB25-IGFR-B8	GSLDESFYDWFERQLGRGRGSTGGPVLSDHQMVAGSRVATQ	10.8	13.2	12.0	1.1	0.9	
RP9NPB25-IGFR-D10	GSLDESFYDWFERQLGVGKGGGHVRNTRKPLGSRGDGVAGY	11.1	13.1	8.5	1.5	0.6	
RP9NPB25-IGFR-F9	GSLDESFYDWFERQLGDPWSRERVGRKEGGRVGFGWHAGVG	11.4	13.0	11.9	1.1	0.9	
RP9NPB25-IGFR-G5	GSLDESFYDWFERQLGISGHIGRDGRQDCLAAGGDPRRTTC	11.5	12.8	12.1	1.1	0.9	
RP9NPB25-IGFR-G4	GSLDESFYDWFERQLGSAVSSVPAGHKDISRQDVGEKSRAG	11.1	12.7	11.0	1.2	0.9	
RP9NPB25-IGFR-D4	GSLDESFYDWFERQLGHSKRCSSVRRTGLVADCADGGHLGY	11.0	12.6	11.3	1.1	0.9	
RP9NPB25-IGFR-D12	GSLDESFYDWFERQLGSWDARACQGSDQGCRVYSETRDLGR	10.0	12.4	8.7	1.4	0.7	
RP9NPB25-IGFR-B3	GSLDESFYDWFERQLGGLAHEGRNLGGSPSDAGGGKHDVEA	10.0	12.3	9.4	1.3	0.8	
RP9NPB25-IGFR-E8	GSLDESFYDWFERQLGTAEAGVGHNLGRGQ	10.8	12.2	6.7	1.8	0.5	
RP9NPB25-IGFR-E3	GSLDESFYDWFERQLGVSARPSGRVKRDHPTGTNGRGLVRD	10.5	12.2	12.6	1.0	1.0	
RP9NPB25-IGFR-B11	GSLDESFYDWFERQLGMQGTAINRTYGPEEEAVRSGGYGPL	9.8	12.1	8.6	1.4	0.7	
RP9NPB25-IGFR-E12	GSLDESFYDWFERQLGAAGSDEADGGSTTVRPSSMLPRTQS	9.7	12.0	10.0	1.2	0.8	
RP9NPB25-IGFR-B2	GSLDESFYDWFERQLGSESSTRRGGWHPVRNSALSERG	10.0	12.0	10.9	1.1	0.9	
RP9NPB25-IGFR-F5	GSLDESFYDWFERQLGRGHDEIHEAGRQGAVLTDSGVRPHV	10.3	11.9	9.7	1.2	0.8	
RP9NPB25-IGFR-B5	GSLDESFYDWFERQLGESRYERRPVRGRSGMNGLVDSGLSD	10.2	11.9	10.7	1.1	0.9	
RP9NPB25-IGFR-F4	GSLDESFYDWFERQLGLAVRSDRAGRNTPRVKYGTRSAVAT	10.5	11.8	11.1	1.1	0.9	
RP9NPB25-IGFR-D7	GSLDESFYDWFERQLGAPERVTGSRGELRNRDLQGRDAYWR	9.9	11.7	9.9	1.2	0.8	
RP9NPB25-IGFR-F1	GSLDESFYDWFERQLGHVGLPLSRGAGTRVGTSQGIGVAGP	10.1	11.6	9.7	1.2	0.8	
RP9NPB25-IGFR-D3	GSLDESFYDWFERQLGGVVEDGGKGRGSRSRKPGGKLPRSG	9.9	11.6	11.4	1.0	1.0	
RP9NPB25-IGFR-G3	GSLDESFYDWFERQLGSRADAPMEHPARWEDRGRVGPGLQD	11.1	11.5	7.8	1.5	0.7	
RP9NPB25-IGFR-A6	GSLDESFYDWFERQLGRAGPGENGVSGSPGAAVGMGGGSIG	9.6	11.5	11.2	1.0	1.0	
RP9NPB25-IGFR-G9	GSLDESFYDWFERQLGNGEGVGHPPNAMQGVIGSAQRAKDS	10.2	11.4	8.0	1.4	0.7	

FIG. 55A

Clone #	Sequence	E-Tag	IGFR	IR	IGFR/IR	IR/IGFR	SEQ ID NO:
RP9NPB25-IGFR-F3	GSLDESFYDWFERQLGA.RTSPPISSCRDRGRSGNMRRRDC	9.8	11.4	10.5	1.1	0.9	
RP9NPB25-IGFR-F6	GSLDESFYDWFERQLGAGRERGSGLSDRERQSGIGGGQKGM	9.8	11.3	10.1	1.1	0.9	
RP9NPB25-IGFR-G7	GSLDESFYDWFERQLGRRQPDSESLDDQAGWVSRIHKGGPK	10.2	11.3	10.2	1.1	0.9	
RP9NPB25-IGFR-A5	GSLDESFYDWFERQLGKPLTSAGRNHNAGCETLGSCLMDRS	8.9	11.1	4.6	2.4	0.4	
RP9NPB25-IGFR-F2	GSLDESFYDWFERQLGQSVDSSMPSVDHPQSMQRGRVGFGG	10.1	10.7	5.3	2.0	0.5	
RP9NPB25-IGFR-D1	GSLDESFYDWFERQLGGDSAGTARGSGRGLSAGADGVAIVG	8.6	10.7	6.7	1.6	0.6	
RP9NPB25-IGFR-C5	GSLDESFYDWFERQLGGGVGIQPVPRDGQFDSGGGGAESSG	10.4	10.7	9.0	1.2	0.8	
RP9NPB25-IGFR-C3	GSLDESFYDWFERQLGTSLAFLRGGDEGWRRGGQAPSAANR	11.5	10.6	7.4	1.4	0.7	
RP9NPB25-IGFR-F10	GSLDESFYDWFERQLGARVDGLSARGATCPTSGGCGVLQPP	8.9	10.4	7.2	1.5	0.7	
RP9NPB25-IGFR-B4	GSLDESFYDWFERQLGPEDRPVGGAIVGQGFFNGVVTIDSA	9.6	10.3	7.2	1.4	0.7	
RP9NPB25-IGFR-E9	GSLDESFYDWFERQLGTYSQGVDRDTSRQ	11.3	10.3	7.1	1.4	0.7	
RP9NPB25-IGFR-E5	GSLDESFYDWFERQLGNSVTDRAHATGLAGKQDQSVDIEVT	11.3	10.1	6.5	1.6	0.6	
RP9NPB25-IGFR-B7	GSLDESFYDWFERQLGLRGQGDAVGRDQTGFRDQGRGSHGR	8.6	10.1	9.5	1.1	0.9	
RP9NPB25-IGFR-G10	GSLDESFYDWFERQLGLPQSGVPGSSGGRGRTSSSMRHGVS	8.8	9.9	8.8	1.1	0.9	
RP9NPB25-IGFR-C4	GSLDESFYDWFERQLGDGRQLGCQGYRAEVDMEGQPRGGCG	9.8	9.8	8.6	1.1	0.9	
RP9NPB25-IGFR-D8	GSLDESFYDWFERQLGTVRGESVVRDAQSPVDSPPGGVARS	8.9	9.6	6.5	1.5	0.7	
RP9NPB25-IGFR-D11	GSLDESFYDWFERQLGAVGGDVGGYREAPRGWGARWLQGAD	8.0	9.4	8.2	1.2	0.9	
RP9NPB25-IGFR-A4	GSLDESFYDWFERQLGVVVRREGNEV	7.7	9.3	7.7	1.2	0.8	
RP9NPB25-IGFR-A10	GSLDESFYDWFERQLGDVGGRGRRGGS	7.5	9.2	9.0	1.0	1.0	
RP9NPB25-IGFR-A11	GSLDESFYDWFERQLGSKPVRLGRGQERRDKPGVEEGGRAA	7.3	9.0	7.4	1.2	0.8	
RP9NPB25-IGFR-B1	GSLDESFYDWFERQLGARVGGMRGQPMLDPRMGPGETQVPS	7.3	8.0	5.9	1.4	0.7	
RP9NPB25-IGFR-D6	GSLDESFYDWFERQLGQTAPVGDASRTKGALQPVGGWYVRG	9.0	7.7	4.7	1.6	0.6	
RP9NPB25-IGFR-G8	GSLDESFYDWFERQLGHVPRAAHVGAQLGSRGPGSLDSLGV	6.0	6.8	4.0	1.7	0.6	
RP9NPB25-IGFR-A9	VEWQLVTVAAGEEGPNLLGVSFYSCLESLVNGGAERSDGQWEGCR	9.4	5.3	1.5	3.5	0.3	
RP9NPB25-IGFR-C12	GSLDESFYDWFERQLGAGDRGQSRTTCMQRNREGAAESCTV	0.9	1.0	0.9	1.1	0.9	

FIG. 55B

RP30-NPB20-vs-IGFR

Clone #	Sequence	E-Tag	IGFR	IR	IGFR/IR	IR/IGFR	SEQ ID NO:
Design	SFYSCLESLVNGGAERSDGQWEGCRXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--	
RP30NPB20-IGFR-E9	SFYSCLESLVNGGAKRSDGQWEGCRSSGWGLSFSGPRGQANSGSG	17.7	19.2	1.1	17.3	0.1	
RP30NPB20-IGFR-E11	SFYSCLESLVNGGAERSDGQWEGCRGQGGGLSTMGFPGLGQESGV	17.1	18.5	1.3	14.4	0.1	
RP30NPB20-IGFR-G12	SFYSCLESLVNGGAERSDGQWEGCRKRHVSASSRGEDRLVGVGSA	15.7	17.5	1.8	9.8	0.1	
RP30NPB20-IGFR-E8	SFYSCLESLVNGGAERSGEGCRGAGGKRMGIDAEGAFGWGVP	17.4	17.1	1.2	14.5	0.1	
RP30NPB20-IGFR-E5	SFYSCLESLVNGGAERSDGQWEGCRSRGQGSVRLRNGGIGRFKVP	15.9	17.1	1.8	9.7	0.1	
RP30NPB20-IGFR-F11	SFYSCLESLVNGGAERSDGQWEGCRFSTSRSADSAGGPGVPWGGS	16.7	14.9	1.6	9.5	0.1	
RP30NPB20-IGFR-F7	SFYSCLESLVNGGAERSDGHWEGCRRAPGHAWSVGHGVRDRGSTQ	14.9	14.9	2.0	7.5	0.1	
RP30NPB20-IGFR-D6	SFYSCLESLVNGGAERSDGQWEGCRAGGGVRQMAGGFSGQSNRMH	14.2	14.8	3.3	4.5	0.2	
RP30NPB20-IGFR-F12	SFYSCLESLVNGGAERSDGQWEGCRARNGRDAALDGAADLIQGMQ	12.7	14.7	1.0	14.3	0.1	
RP30NPB20-IGFR-D2	SFYSCLESLVNGGAERSDGQWEGCRRRGWVEDHEDGGGMLGSLAQ	13.1	14.7	2.1	7.0	0.1	
RP30NPB20-IGFR-D3	SFYSCLESLVNGGAERSDGQWEGCRGTVGGANPRGGATWIGTPLQ	12.8	14.6	6.5	2.3	0.4	
RP30NPB20-IGFR-D5	SFYSCLESLVNGGAERSDGQWEGCRVEGGVHGLEPGGSNLGHGGL	14.8	14.4	2.9	4.9	0.2	
RP30NPB20-IGFR-H2	SFYSCLESLVNGGAERSDGQWEGCRGVPGGVVGQASGQWAESGVE	13.6	14.1	1.4	10.3	0.1	
RP30NPB20-IGFR-E6	SFYSCLESLVNGGAERSDGQWEGCRGSGITGDLRTRVGSGRDAAI	15.3	13.7	1.5	9.2	0.1	
RP30NPB20-IGFR-B3	SFYSCLESLVNGGAERSDGQWEGCRGRIAP	12.5	13.4	1.5	8.8	0.1	
RP30NPB20-IGFR-F1	SFYSCLESLVNGGAEDGQWEGCRGHRTSQFRYGRHLGRTGGSV	12.7	13.2	1.1	12.4	0.1	
RP30NPB20-IGFR-E3	SFYSCLESLVNGGAERSDGQWEGCRTRAGGQVRYAASGDGPLRAA	13.6	13.0	1.2	11.0	0.1	
RP30NPB20-IGFR-E12	SFYSCLESLVNGGAERSDGQWEGCRSRATLGDVEVRGSGGFVNGM	10.4	12.6	0.8	16.3	0.1	
RP30NPB20-IGFR-C10	SFYSCLESLVNGGAERSDGQWEGCREAPGDFVVGFGGRVAQGQGI	13.4	12.6	1.9	6.6	0.2	
RP30NPB20-IGFR-E4	SFYSCLESLVNGGAERSDGQWEGCRVGVGRLQGGPALSM	14.8	12.3	1.0	12.9	0.1	
RP30NPB20-IGFR-F2	SFYSCLESLVNGDAERSDGQWEGCHGASSGRGVRQGTTGGRSWY	14.2	12.1	1.8	6.8	0.1	
RP30NPB20-IGFR-E7	SFYSCLESLVNGGAERSDGQWEGCRFSGSGSGYTDVRGSTLGGTA	15.7	12.0	1.1	10.5	0.1	
RP30NPB20-IGFR-D8	SFYSCLESLVNGGAERSDGQWEGCRRGGLRSWGDWRETAGPDLGA	11.2	11.8	2.5	4.7	0.2	
RP30NPB20-IGFR-G3	SFYSCLESLINGGAERSDGQWEGCRPAPGRAGGRGGIQSGGGARV	10.0	11.8	3.3	3.6	0.3	
RP30NPB20-IGFR-F8	SFYSCLESLVNGGAERSDGQWEGCRRGFDAWGMGWSTAGSRGGVS	13.5	11.6	0.8	13.8	0.1	
RP30NPB20-IGFR-F10	SFYSCLESLVNGGAERSDSQWEGCRGSGALSGLFNGLPVLAGDHG	13.1	11.6	1.0	11.2	0.1	
RP30NPB20-IGFR-F9	SFYSCLESLVNGGAERSDGQWEGCRSSGPQVSAGGGPGASSAAGG	13.2	11.3	1.0	10.8	0.1	
RP30NPB20-IGFR-G11	SFYSCLESLVNGGAERSDGQWEGCRRGWDIVLDGRVQNGSAGHGI	10.6	11.0	2.2	5.0	0.2	
RP30NPB20-IGFR-B12	SFYSCLESLVNGGAERSDGQWEGCRGSGVDVGLPVGPGGSPVSLS	10.4	11.0	2.5	4.4	0.2	
RP30NPB20-IGFR-G7	SFYSCLESLVNGGAERSDGQWEGCRSYHGHREWPLQDGGRDDSVI	11.1	10.8	1.8	6.0	0.2	
RP30NPB20-IGFR-A7	SFYSCLESLVNGGAERSDGQWEGCRRGGGIGGAVGTDGRTQVGIR	9.9	10.8	2.0	5.4	0.2	
RP30NPB20-IGFR-G6	SFYSCLESLVNGGAERSDGQWEGCRSFRPRTDSGEKGTPAAGGSI	10.9	10.8	2.5	4.4	0.2	
RP30NPB20-IGFR-C4	SFYSCLESLVNGGAERSDGQWEGCRRPWAPSGADVEIFGCGGNVC	12.4	10.5	1.4	7.4	0.1	
RP30NPB20-IGFR-G8	SFYSCLESLVNGGAERSDGQWEGCRDHAFGEGGLAQDIGSRPRGG	11.4	10.4	1.9	5.5	0.2	
RP30NPB20-IGFR-G4	SFYSCLESLVNGGAERSDGQWEGCRGGLSAGPYGGSGAVALEGGN	9.5	10.0	1.4	7.1	0.1	
RP30NPB20-IGFR-H12	SFYSCLESLVNGGAERSDGQWEGCRAGGVEVRNNVRPGRSIRPRD	12.0	10.0	2.2	4.6	0.2	

FIG. 56A

Clone #	Sequence	E-Tag	IGFR	IR	IGFR/IR	IR/IGFR	SEQ ID NO:
RP30NPB20-IGFR-D10	SFYSCLESLVNGGAERSDGQWEGCRRGQAPGGVFSRGGGRAASAL	11.3	9.8	3.0	3.3	0.3	
RP30NPB20-IGFR-A5	SFYSCLESLVNGGAERSDGQWEGCRTSGQGQLGSRKTTSEGAVHA	14.2	9.5	1.5	6.5	0.2	
RP30NPB20-IGFR-A11	SFYSCLESLVNGGAERSDGQWEGCRGFGGVVGAVSGGPGTPAGSV	8.8	9.5	1.6	5.7	0.2	
RP30NPB20-IGFR-D7	SFYSCLESLVNGGAERSDGQWEGCRRASWVRGHDQATFGYGVGGT	12.6	9.5	2.4	4.0	0.3	
RP30NPB20-IGFR-C3	SFYSCLESLVNGGAERSDGQWEGCRIGGRRAPGHGQVAQTHLRGV	12.9	9.4	1.9	5.0	0.2	
RP30NPB20-IGFR-C11	SFYSCLESLVNGGAERSDGQWEGCRRFTRDGLVGLQGSESGGELV	9.9	9.2	1.2	7.6	0.1	
RP30NPB20-IGFR-G9	SFYSCLESLVNGGAERSDGQWEGCRSSSSSGAPWMGWAGFGLHGN	10.0	9.2	3.3	2.8	0.4	
RP30NPB20-IGFR-D1	SFYSCLESLVNGGAERSGRWEGCRGDVVGKTPDSHEVARSELVV	7.6	9.2	3.3	2.7	0.4	
RP30NPB20-IGFR-C5	SFYSCLESLVNGGAERSDGQWEGCRGGNAGQGPRGGAGHRRGA	9.4	9.2	3.8	2.4	0.4	
RP30NPB20-IGFR-H6	SFYSCLESLVNGGAERSDGQWEGCRWLGRGRSSGMSVGTGARLSS	10.6	8.6	1.1	7.9	0.1	
RP30NPB20-IGFR-D9	SFYSCLKSLVNGGAERSDGQWEGCRGVGPPFTNAPEVIRQGGGIS	7.7	8.6	1.1	7.5	0.1	
RP30NPB20-IGFR-D12	SFYSCLESLVNGGAERSDGQWGGCRRGKELSGAYQWKGAPSLSWL	8.2	8.6	1.2	7.3	0.1	
RP30NPB20-IGFR-C7	SFYSCLESLVNGGTERSDGQWEGCRGPVFGSAGHQGDSTLAGGVE	8.2	8.6	3.0	2.9	0.3	
RP30NPB20-IGFR-$F6_2$	SFYSCLESLVNGGAERSDGQWEGCRTSIPEGGLKPRGSPGGALPI	17.9	8.5	1.4	6.1	0.2	
RP30NPB20-IGFR-C6	SFYSCLESLVNGGAERSDGQWEGCRGSLWQVGQRGRVLGMGGVSS	8.4	8.4	3.1	2.7	0.4	
RP30NPB20-IGFR-D4	SFYSCLESLVNGGAERSDGQWEGCRSRQGLGPGMEEVSGPTSGNH	15.1	8.3	2.0	4.2	0.2	
RP30NPB20-IGFR-H3	SFYSCLESLVNGGAERSDGQWEGCRGARRRQWPAGKDPSRRGDVP	9.1	8.2	1.5	5.5	0.2	
RP30NPB20-IGFR-B8	SFYSCLESLVNGGAERSDGQWEGCRGRKDGVWASSVPPRTQRGGH	6.8	7.9	2.4	3.3	0.3	
RP30NPB20-IGFR-G5	SFYSCLESLVNGGTERSDGQWEGCRAAQAFLGESGMNGVSWYGEQ	14.7	7.5	1.4	5.3	0.2	
RP30NPB20-IGFR-B7	SFYSCLESLVNGGAERSDGQWEGCRVSGGWPANLDSHGARGTIQA	10.3	7.4	2.0	3.6	0.3	
RP30NPB20-IGFR-B2	SFYSCLESLVNGGAERSDGQWEGCRGQKVLRSGRPVFLEGADTGT	9.0	7.4	3.1	2.4	0.4	
RP30NPB20-IGFR-B10	SFYSCLESLVNGGAERSDGQWEGCRTTGLLWGGDPPRGRSAGIAF	9.3	7.0	1.8	3.9	0.3	
RP30NPB20-IGFR-F3	SFYSCLESLVNGGAERSDGQWEGCRGSWQLRADWIGRGGRTGPDG	12.0	6.8	2.4	2.8	0.4	
RP30NPB20-IGFR-B6	SFYSCLESLVNGGAERSDGQWEGCRQGREFFQFSRGGSGSYVGGV	6.0	6.6	2.2	3.0	0.3	
RP30NPB20-IGFR-G10	SFYSCLESLVNSGAERSDGQWEGCRVGWDGHVRGEPLSLGVAQIM	12.5	6.4	1.2	5.5	0.2	
RP30NPB20-IGFR-C9	SFYSCLESLVNGGAERSDGQWEGCRISAATAQLGYGLPESGTRWR	7.8	6.3	1.4	4.6	0.2	
RP30NPB20-IGFR-B1	SFYSCLESLVNGGAERWEGCRRQGQHFFEGRVGGGANHGGV	5.5	6.3	2.4	2.6	0.4	
RP30NPB20-IGFR-B5	SFYSCLESLVNGGAERSDGQWEGCRTAGVSSDGSGVGFVVLGDNA	10.5	6.2	1.8	3.5	0.3	
RP30NPB20-IGFR-H9	SFYSCLESLVNGGAERSDGQWEGCRWLAVAATGGGREADVGLRLS	14.0	6.1	1.2	5.1	0.2	
RP30NPB20-IGFR-H5	SFYSCLESLVNGGAERSDGQWEGCRQSGVSSNSAGVRAQVGSMGG	8.0	6.0	1.5	3.9	0.3	
RP30NPB20-IGFR-C8	SFYSCLESLVNGGAERSDGQWEGCRVGGQVGRTVFPQGGFPRVVA	9.0	5.7	1.0	5.6	0.2	
RP30NPB20-IGFR-H7	SFYSCLESLVNGGAERSDGQWEGCRFFESAGVQVQGPLGTGRGSE	7.3	5.4	1.4	4.0	0.2	
RP30NPB20-IGFR-B9	SFYSCLESLVNGGAERSDGQWEGCRLSWGQGRLVLGGRDQTRGLQ	7.5	5.3	0.9	5.6	0.2	
RP30NPB20-IGFR-B11	SFYSCLESLVNGGERSDGQWEGCRTITTEQRQRGGPDRAGAGRS	9.2	5.2	1.3	3.8	0.3	
RP30NPB20-IGFR-H11	SFYSCLESLVNGGAERSDGQWEGCRGAVGAPRGGGVDVLSQSQLR	7.4	5.2	1.7	3.1	0.3	
RP30NPB20-IGFR-D11	SFYSCLESLVNGGAERSDGQWEGCRLRILDRTGRQGVTDPVHGPV	13.6	5.1	2.4	2.2	0.5	
RP30NPB20-IGFR-H4	SFYSCLESLVNGGAERSDGQWEGCRTSLAIQVQDREVAVRGRGDL	9.2	3.4	1.1	3.1	0.3	
RP30NPB20-IGFR-B4	SFYSCLESLVNGGAERSDGQWEGCRLQHGPVLAKVPPGVGRINFF	9.9	3.1	1.0	3.0	0.3	
RP30NPB20-IGFR-C12	SFYSCLESLVNGGAERSDGQWEGCRDVPPLPQLWGEPWRVPGDNR	0.9	0.9	0.8	1.0	1.0	

FIG. 56B

NPB20RP30 VS IGFR

Clone #	Sequence	E-Tag	IGFR	IR-Fc	IGFR/IR	IR/IGFR	SEQ ID NO:
Design	XXXXXXXXXXXXXXXXXXXXSFYSCLESLVNGGAERSDGQWEGCR						
NPB20RP30-IGFR-H12	DGDVGGRVIARVGSFYSCLESLVNGGAERSDGQWEGCR	10.9	4.3	1.3	3.5	0.3	
NPB20RP30-IGFR-H5	DGRQPRVAGGSANRVVAYGVSFYSCLESLVNGGAERSDGQWEGCR	10.6	10.4	1.9	5.5	0.2	
NPB20RP30-IGFR-F11	EDLVAAFGAGEGGGHSFYSCLESLVNGGAERSDGQWEGCR	13.8	2.7	1.4	2.0	0.5	
NPB20RP30-IGFR-A10	EGISMRLEVSFYSCLESLVNGGAERSDGQWEGCR	10.8	3.0	1.0	2.9	0.3	
NPB20RP30-IGFR-D7	EKVIAIKASSGSQKPPLDPSSFYSCLESLVNGGAERSDGRWEGCR	15.5	9.4	2.0	4.7	0.2	
NPB20RP30-IGFR-H9	ELGLRQPMVVSGGSPESGRRSFYSCLESLVNGGAERSDGQWEGCR	13.1	4.2	1.5	2.7	0.4	
NPB20RP30-IGFR-G8	FFGRSFYSCLESLVNGGAERSDGQWEGCR	14.7	14.4	1.5	9.9	0.1	
NPB20RP30-IGFR-H8	FLKNAYGGPGKESDRARLVTSFYSCLESLVNGGAERSDGQWEGCR	12.0	11.2	1.2	9.5	0.1	
NPB20RP30-IGFR-E10	GGATSSGGITSEFASMAGGLSFYSCLESLVNGGAERSDGQWEGCR	14.8	3.8	2.2	1.8	0.6	
NPB20RP30-IGFR-A5	GGGPSTVQSVWSGSFYSCLESLVNGGAERSDGQWEGCR	4.3	1.3	1.0	1.3	0.8	
NPB20RP30-IGFR-F3	GGNGKTRSFYSCLESLVNGGAERSDGQWEGCR	15.2	13.1	2.3	5.6	0.2	
NPB20RP30-IGFR-E4	GGSLYVDGRGGSGDEPDGAVSFYSCLESLVNGGAERSDGQWEGCR	11.4	5.5	1.8	3.0	0.3	
NPB20RP30-IGFR-E7	GKILNDIPVTGRRNGFADNRSFYSCLESLVNGGAERSDGQWEGCR	16.9	11.6	3.7	3.2	0.3	
NPB20RP30-IGFR-D4	GLGGVTRSDVPVGLRRSNSISFYSCLESLVNGGAERSDGQWEGCR	15.6	14.9	5.6	2.7	0.4	
NPB20RP30-IGFR-E8	GLIRWIADEVKASRVTPLTSSFYSCLESLVNGGAERSDGQWEGCR	13.2	5.8	1.2	4.7	0.2	
NPB20RP30-IGFR-G10	GRASWKDGSNGSVPVGSGRVSFYSCLESLVNGGAERSDGQWEGCR	12.6	11.7	3.0	3.9	0.3	
NPB20RP30-IGFR-G9	GSNSSSQGRVGLRGSASDGVSFYSCLESLVNGGAERSDGQWEGCR	14.8	14.7	2.3	6.3	0.2	
NPB20RP30-IGFR-G3	HAGSLGMPGASEGRFTRRLLSFYSCLESLVNGGAERSDGQWEGCR	13.6	14.8	2.8	5.2	0.2	
NPB20RP30-IGFR-H7	HRAGPREEFYSGFLEIADGRSFYSCLESLVNGGAERSDGQWEGCR	11.9	6.4	2.2	2.9	0.3	
NPB20RP30-IGFR-D5	LETFVGAGHAT.KINWRGPTSFYSCLESLVNGGAERSDGQWEGCR	15.0	7.2	2.4	3.0	0.3	
NPB20RP30-IGFR-F7	LGQVRVGDMYRTVRFSAGYVSFYSCLESLVNGGAERSDGQWEGCR	16.1	8.6	1.4	6.4	0.2	
NPB20RP30-IGFR-H11	LPLGSH.GSPLGVIARV.GGSFYSCLESLVNGGAERSDGQWEGCR	11.1	9.2	1.7	5.4	0.2	
NPB20RP30-IGFR-D3	LRTNPLHTF.GGVSGPGQSFYSCLESLVNGGAERSDGQWEGCR	12.3	11.4	3.9	2.9	0.3	
NPB20RP30-IGFR-A3	LSTRYEYVSSVWSRSVSGSTHSFYSCLESLVNGGAERSDGQWEGCR	10.6	5.4	2.1	2.6	0.4	
NPB20RP30-IGFR-E5	NASLWSPGATDGDGRSNFKHSFYSCLESLVNGGAERSDGQWEGCR	17.5	5.9	1.9	3.1	0.3	
NPB20RP30-IGFR-G11	QFDYHFGLALGGTEIVDRQVSFYSCLESLVNGGAER.WEGCR	7.1	5.5	0.9	6.1	0.2	
NPB20RP30-IGFR-F10	QIVVAARGDVRGGSFYSCLESLVNGGAERSDGQWEGCR	14.1	12.7	3.9	3.3	0.3	
NPB20RP30-IGFR-H6	QLFSFSPDFTSGAIRGGASFYSCLESLVNGGAERSDGQWEGCR	7.8	8.1	2.1	3.9	0.3	
NPB20RP30-IGFR-A6	QRVRVGQPVGGFTRWEPGGNSFYSCLESLVNGGQWEGCR	9.3	9.8	3.8	2.6	0.4	
NPB20RP30-IGFR-H3	R.VAVISIGPESRRGSEVRVSFYSCLESLVNGGAERSDGQWEGCR	11.9	11.4	2.2	5.1	0.2	
NPB20RP30-IGFR-E6	RASNAAFIAMPSISARYAEGSFYSCLESLVNGGAERSDGQWEGCR	16.6	4.4	1.4	3.2	0.3	
NPB20RP30-IGFR-E3	RSFYSCLESLVNGGAERSDGQWEGCR	14.4	10.5	2.5	4.1	0.2	
NPB20RP30-IGFR-D6	SDSPFELHMSPRRDPWVRRGSFYSCLESLVNGGAEHSDGQWEGCR	14.7	16.0	5.2	3.1	0.3	
NPB20RP30-IGFR-E12	SGGAHALTE.FILFATPNRASFYSCLESLVNGGAERSDGQWEGCR	10.2	7.4	1.8	4.1	0.2	
NPB20RP30-IGFR-F9	SHVSPAIPTFNELDSQVMGISFYSCLESLVNGGAERSDGQWEGCR	14.7	3.0	1.6	1.9	0.5	
NPB20RP30-IGFR-F5	SLRVKSDSTSMNPGTGLASISFYSCLESLVNGGAERSDGQWEGCR	16.9	13.1	2.5	5.1	0.2	
NPB20RP30-IGFR-A11	SVFVGFRQVSVGGPSFGHVFSFYSCLESLVNGGAERSDGQWEGCR	11.5	7.2	2.8	2.6	0.4	

FIG. 57A

Clone #	Sequence	E-Tag	IGFR	IR-Fc	IGFR/IR	IR/IGFR	SEQ ID NO:
NPB20RP30-IGFR-A12	SVVDTRDGVGSLRRSAVSSTSFYSCLESLVNGGAERSDGQWEGCR	13.7	6.9	1.8	3.8	0.3	
NPB20RP30-IGFR-F12	TGFAAMVTARRGSHAVTDDPSFYSCLESLVNGGAERSDGQWEGCR	9.5	9.7	2.4	4.0	0.2	
NPB20RP30-IGFR-G6	TQAGAHRSFTRLHKAMLQSSSFYSCLESLVNGGAERSDGQWEGCR	11.6	7.5	1.0	7.4	0.1	
NPB20RP30-IGFR-G5	TRWGVDSTILGHGRSILVSRSFYSCLESLVNGGAERSDGQWEGCR	14.9	6.5	1.1	5.9	0.2	
NPB20RP30-IGFR-F6	VGPSPRSGGWLGGIGDKSGVSFYSCLESLVNGGAERSDGQWEGCR	16.3	14.7	3.4	4.3	0.2	
NPB20RP30-IGFR-G12	VLRAENSRFSTASDFALSPVSFYSCLESLVNGGAERSDGQWEGCR	16.5	10.5	1.8	5.8	0.2	
NPB20RP30-IGFR-E11	WGGIEDRAFWLALGGRSTTPSFYSCLESLVNGGAERSDGQWEGCR	16.7	14.4	2.4	5.9	0.2	
NPB20RP30-IGFR-F8	WLARGRSEVPSFYSCLESLVNGGAERSDGQWEGCR	12.5	14.0	4.3	3.3	0.3	
NPB20RP30-IGFR-H10	WNATMGSRNKSPGGGSAVEMSFYSCLESLVNGGAERSDGQWEGCR	9.7	12.2	6.1	2.0	0.5	
NPB20RP30-IGFR-E9	WQYDLIGSSSRSGDSRSSRVSFYSCLESLVNGGAERSDGQWEGCR	16.7	13.4	5.7	2.3	0.4	
NPB20RP30-IGFR-H4	WSVATLHHVD.GAAPSPKVLSFYSCLESLVNGGAERSDGQWEGCR	14.3	7.8	2.3	3.4	0.3	
NPB20RP30-IGFR-D9	WVGGRAGSVRLYAVDGVVGGSFYSCLESLVNGGAERSDRQWEGCR	15.1	10.0	2.5	4.0	0.3	

FIG. 57B

D815 vs IGFR

Clone	Sequence	ETAG	IGFR	IR	IGFR/IR	SEQ ID NO:
Design	WLDQEWAWVQCEVYGRGCPS	--	--	--	--	
D815-IGFR-A10	GLDQDRAWLWCEISGHGCLS	36.8	5.7	1.0	5.7	
D815-IGFR-A11	GVDQERAWLWCQISGRGCQS	17.6	5.6	0.5	12.0	
D815-IGFR-$A1_2$	GLAEERAWLWCQISGRGCLS	37.1	16.6	1.3	12.7	
D815-IGFR-A2	GLEEERAWLWCQISGRGCLS	38.0	15.3	0.9	16.3	
D815-IGFR-A3	GLDEERAWLWCEISGRGCLS	39.6	7.2	1.0	7.3	
D815-IGFR-A4	SLEQERAWLWCQISGRGCSS	39.8	9.4	1.1	8.8	
D815-IGFR-A5	SLDQERAWLWCQISGRGCLS	42.5	10.5	1.0	10.2	
D815-IGFR-A6	WLDQERAWLWCEISGLGCPS	35.1	4.2	0.9	4.7	
D815-IGFR-$A8_3$	GPDQVRAWLWCEISGRGCLS	36.5	10.3	1.2	8.8	
D815-IGFR-A9	QLDEERAWLWCEISGLGCLR	34.1	2.2	1.1	2.0	
D815-IGFR-B12	GLDQERAWLWCEISGHGCLP	31.8	3.5	0.9	3.9	
D815-IGFR-B1	WLDQERAWLWCEISGQGCLS	30.9	5.7	1.2	4.9	
D815-IGFR-B3	GLDEERAWLWCQISGRGCLS	31.6	12.5	0.9	13.7	
D815-IGFR-B4	WLDKERAWLWCEISGHGCLS	29.8	2.0	0.9	2.2	
D815-IGFR-B5	QLAQERAWLWCQISGRGCLS	31.0	2.9	0.9	3.1	
D815-IGFR-B6	GLDEERAWLWCVISGGGCVP	30.4	2.4	0.9	2.7	
D815-IGFR-B8	WLDRERAWLWCEISGLGCLS	30.3	2.4	1.0	2.4	
D815-IGFR-C10	QGDQVRAWLWCQISGRGCLS	22.7	4.4	0.7	6.0	
D815-IGFR-C12	RLDQERAWLWCQISGRGCLS	31.4	2.8	0.9	3.2	
D815-IGFR-$C3_2$	GPDQVRAWLWCEISGRGCLS	32.3	9.9	1.0	9.9	
D815-IGFR-C4	GLDQDRAWLWCEISGRGCRS	32.5	4.2	1.0	4.4	
D815-IGFR-C5	WLDRERAWLWCEISGYGCLS	25.9	1.5	0.8	1.9	
D815-IGFR-C6	WLDRERAWIWCEMTGRGCLS	26.6	2.5	0.8	3.1	
D815-IGFR-C7	GLAEDRAWLWCEISGRGCVS	32.6	12.4	0.9	13.8	
D815-IGFR-C8	QVDEVRAWLWCEISGRGCLS	32.6	3.8	1.0	3.8	
D815-IGFR-D10	GVEQERAWLWCEISGLGCLS	29.5	10.7	0.9	11.5	
D815-IGFR-D11	GPEQDRAWLWCQISGRGCLS	26.5	4.0	0.9	4.7	
D815-IGFR-D12	SLDQERAWLWCQISGRGCLS	25.3	4.7	0.8	5.8	
D815-IGFR-D1	GLEEERAWLWCEISGLGCLS	31.6	17.1	0.9	18.5	
D815-IGFR-D5	SLDEDRAWLWCQISGRGCLS	33.3	14.6	0.9	16.1	
D815-IGFR-D6	WLDRERAWLWCEISGLGCPS	24.6	3.0	0.8	3.9	
D815-IGFR-D7	WLDRERAWLWCEISGLGCES	23.1	4.2	0.8	5.2	

FIG. 58A

Clone	Sequence	ETAG	IGFR	IR	IGFR/IR	SEQ ID NO:
D815-IGFR-D8	WLDEERAWLWCEISGHGCLS	30.2	2.7	1.0	2.7	
D815-IGFR-D9	RVDQVRAWLWCEISGLGCPS	29.3	12.5	1.0	13.0	
D815-IGFR-E12	GLDEERAWLWCQITGRGCLS	26.8	7.1	0.7	9.8	
D815-IGFR-E1	WLDQERAWLWCEISGLGCLS	27.2	2.9	0.8	3.5	
D815-IGFR-E2	SLDQERAWLWCQISGRGCLT	33.2	6.9	1.0	7.1	
D815-IGFR-E3	GLDQTRAWLWCQISGRGCLF	24.9	2.8	0.8	3.7	
D815-IGFR-E4	GPDELRAWLWCEISGRGCLS	33.6	18.1	1.0	17.5	
D815-IGFR-E6	QLDQVRAWLWCEISGQGCRS	20.8	3.0	0.9	3.5	
D815-IGFR-E9	WLDRERAWLWCEISGLGCLA	23.9	1.9	0.9	2.1	
D815-IGFR-F10	GHDQVRAWLWCQISGSGCLS	11.7	2.4	0.4	6.5	
D815-IGFR-F11	WLDRERAWLWCEISGEGCLS	25.6	4.2	0.8	5.5	
D815-IGFR-F5	GLDQDRAWLWCQISGRGCLS	32.2	7.7	1.0	7.7	
D815-IGFR-G10	GLDQERAWLWCQISGRGCMS	32.0	5.2	1.0	5.3	
D815-IGFR-G1	QLDRDRAWLWCEISGLGCLS	31.1	3.6	1.0	3.8	
D815-IGFR-G2	GVDEERAWLWCQISGLGCMF	33.1	14.0	1.0	13.8	
D815-IGFR-G3	SLEQERAWLWCEISGIGCMS	30.3	11.8	1.0	12.2	
D815-IGFR-G4	GLDQERAWLWCQISGRGCLS	32.3	6.7	1.0	6.6	
D815-IGFR-G5	WLDEERAWLWCEISGQGCLS	31.2	6.2	1.0	6.0	
D815-IGFR-G6$_3$	SPEQIRAWLWCEISGIGCVS	28.6	15.9	0.9	16.8	
D815-IGFR-G7	LLDQERAWLWCEISGQGCQS	26.3	2.6	1.0	2.5	
D815-IGFR-G8	GLEQERAWLWCQISGRGCLS	31.7	6.2	1.0	6.2	
D815-IGFR-H2$_3$	WLDRERAWLWCEISGLGCLS	24.3	2.4	0.7	3.2	
D815-IGFR-H4	WLDQERAWLWCEISGQGCLS	32.0	4.8	0.9	5.3	
D815-IGFR-H7	SLDQERAWLWCEISGLGCVS	30.1	6.0	0.9	6.3	

FIG. 58B

RP6-D815 VS IGFR

Clone #	Sequence	E-Tag	IGFR	LDH	Sp/Ir SEQ ID NO:
Design	TFYSCLASLLTGTPQPNRGPWERCRWLDQERAWLWCEISGRGCLS	--	--	--	--
RP6-D815-IGFR-4-D7	GSPQPERGLRERSRWLDQERAWLWCEISGRGCLS	20.0	18.3	1.1	17.1
RP6-D815-IGFR-4-B11	SFYSCLASLVTGSSQPKRGPWGCLS	17.6	17.6	1.0	16.9
RP6-D815-IGFR-4-C9	SRLFGSAQLNGGAGERSRWLDQERAWLWCEISGRGCLP	19.5	15.9	1.0	16.2
RP6-D815-IGFR-4-G11	WERSPWLEQERAWLWCEISGRGCVS	16.4	15.7	1.0	16.4
RP6-D815-IGFR-4-F7	SPRERGRSLDLDRAWLWCVISGRDCGA	20.8	14.5	1.2	12.1
RP6-D815-IGFR-4-E9	SFYCGLAALLTGTPADRWPWDASGGEISGRGCLS	17.3	13.0	1.0	13.2
RP6-D815-IGFR-4-F2	TLYTSLDSLQSGTPQPSRGPSERRRWLEQERAWLWCQISGSGCLS	16.9	12.3	1.1	11.2
RP6-D815-IGFR-4-H6	SFYDCLATLLTGTPQPTRGSGERIGERGCRS	14.5	11.8	1.1	11.2
RP6-D815-IGFR-4-F9	SERSRWLLQERAWLWCEISGRGCVA	15.5	11.1	1.0	10.9
RP6-D815-IGFR-4-G5	SFYSCLDALLNGGRKPNSGAGELCSWRAQERARPASEICGRGCRT	9.2	10.2	1.1	9.1
RP6-D815-IGFR-4-F10	TERRLWLDRERAWLWCEISGRGCLS	18.0	10.0	1.0	10.4
RP6-D815-IGFR-4-H8	SFYSCLASLLTGTPDENRGGWERFRCLDQAGDGLWGEPSGRGLVS	16.6	9.9	1.1	8.8
RP6-D815-IGFR-4-C4	TERRLWLDRERAWLWCEISGRGCLS	19.3	9.7	1.1	8.9
RP6-D815-IGFR-4-D11	SFYSCLASLVTGTRQPSRGCLS	19.6	9.6	1.0	9.6
RP6-D815-IGFR-4-A9	SWYSGGWERRSWLDQERAWLWCEISGRGCPA	14.1	8.8	1.0	8.8
RP6-D815-IGFR-4-H11	SFYSCLGALLAGPPQPGRGLWERCRWRDEGGAWQGCENSGRACLS	6.4	8.4	1.0	8.7
RP6-D815-IGFR-4-F1	HSEIAVASLRSRQLEQERAWLWCEISGRGCLA	11.5	8.1	1.1	7.1
RP6-D815-IGFR-4-G12	SFYSCLTSLLSGADGGPRERCRW	13.1	7.9	0.9	8.6
RP6-D815-IGFR-4-G9	RSYSSLDSWEGGTAQPNRGTLERGGWLDQERAWLWCEISGRCGLA	14.0	7.8	1.4	5.5
RP6-D815-IGFR-4-F12	HVPLVVSVDGARDPIRGPWQRIHWLDEDRAWLWCEISGRGCRS	15.0	7.4	0.9	8.2
RP6-D815-IGFR-4-G2	SFYSCLSSLVTGTAQREPWSWERCRQLDQERAWLGGGLAGRGGLS	15.6	7.2	1.5	4.9
RP6-D815-IGFR-4-D8	SGYSDRWRRLDQDRAWLWCEISGRGCLA	20.1	7.1	1.1	6.4
RP6-D815-IGFR-4-G8	TFYSCLDSLLTATPRRQDGVGLWSEISGRGCRS	18.0	7.1	1.1	6.5
RP6-D815-IGFR-4-B7	NFYSCLASLLSATPQPRGGRGCLS	19.3	6.9	1.0	6.7
RP6-D815-IGFR-4-H9	TFYSCLASLLADKAQPNRGSGEGWREIAGRECLS	16.7	6.9	1.6	4.3
RP6-D815-IGFR-4-B3	RGSSDRSLWLDQDRAWLWCEISGRGCLS	19.0	6.8	1.0	6.5
RP6-D815-IGFR-4-A2	SFYSCLASLVTGTAQSNRGSWERCRGGLY	17.3	6.7	9.5	0.7
RP6-D815-IGFR-4-F3	TLYSGQESMGSGSPQLDRGPGARVRQLDQVRAWLWCEISGHGCLS	14.2	6.7	0.8	8.3
RP6-D815-IGFR-4-C8	SPRERGRSLDLDRAWLWCVISGRDCGA	20.0	6.5	0.9	7.1
RP6-D815-IGFR-4-D1	RGPSEHSRWLERERAWLWCEMSGRGCVS	10.9	6.5	1.0	6.4

FIG. 59A

Clone #	Sequence	E-Tag	IGFR	LDH	Sp/Ir SEQ ID NO:
RP6-D815-IGFR-4-F4	SFYSCLASLMTGEISANGCLS	9.5	6.4	1.0	6.6
RP6-D815-IGFR-4-C2	SFYSCLASLLTATSQPNRGPGDRCERSGGGWLS	11.1	6.1	3.7	1.6
RP6-D815-IGFR-4-H1	KIQAGPASLLTGITQPNGGAWERYRSLDQERAWLWCQISGRGCES	8.8	5.3	5.9	0.9
RP6-D815-IGFR-4-B2	TFASGRAASLNGSPKSDGGPWGRGSSLDEERAWLWCEISGRGCLH	18.6	4.8	0.9	5.6
RP6-D815-IGFR-4-C3	TFYSCLASLLTGSAEGCRGRGEGWRGLEKERACVWGDMAGRGCQS	10.9	4.7	1.0	4.6
RP6-D815-IGFR-4-A4	SFYSCMASLLNGPRERNPGQRECFRGREPGRASRGCELSGRGCVT	13.8	4.3	0.9	4.7
RP6-D815-IGFR-4-E8	TFYSCLASLLADKAQPNRGSGEGWREIAGRECLS	8.7	4.2	1.0	4.3
RP6-D815-IGFR-4-E1	SFYSCLAFLLRGTPQPCNGPGERCRWSDQDRAQLWCEQPGTGSLS	12.7	4.1	1.2	3.3
RP6-D815-IGFR-4-A10	TCYSQVAPLLNGTLLPSRGQGERSRWLDQQRAWLWCEISGRGCLT	5.0	4.0	1.2	3.3
RP6-D815-IGFR-4-A11	SLCSGMACVLNGTAQPNRGLLKGRRWLDQERAWLWCEISGRGCQS	3.4	3.6	0.9	3.8
RP6-D815-IGFR-4-H12	TRNNGLGRWVDQERAWLWCEISGRGCLF	8.8	3.1	0.8	4.0
RP6-D815-IGFR-4-H7	SPRERGRSLDLDRAWLWCVISGRDCGA	6.5	3.1	1.2	2.6
RP6-D815-IGFR-4-B6	SFEQWVASLRAGTPQPQGGPSGRIRSVDQERAWLWCEISGRGCLS	15.9	2.8	1.1	2.5
RP6-D815-IGFR-4-E10	TSYSQQASLPTGTAQPNRRPSERWRWLDQERAWLWCEISGRGCRS	4.1	2.0	1.0	2.1

FIG. 59B

RP6-6-D815 vs IGFR

CLONE#	Sequence	Etag	IGFR	LDH	IGFR/LDH	SEQ ID NO:
Design	TFYSCLASLLTGTPQPNRGPGGSGGSWLDQERAWLWCEISGRGCLS	--	--	--	--	
RP6-6-D815-IGFR-4-H10	SLSAYRGGSGGSGGDRERAWLWCEISGHGCVS	14.9	17.7	1.3	14.1	
RP6-6-D815-IGFR-4-D11	SFYSCLAHLMTGPPQPKGGPWERYCGGQS	12.7	17.4	1.4	12.8	
RP6-6-D815-IGFR-4-B12	QLDQGFYSCLEYLRTGAPQTDGGPWERCR	11.7	17.4	1.3	13.7	
RP6-6-D815-IGFR-4-D9	SFESYLGPLLTSTTQPNRDPRERGRGGSGGSWLEQERAWLWCEIAGRGCLS	13.4	17.3	1.6	10.7	
RP6-6-D815-IGFR-4-C9	STVSRGGGGSWLDQQRAWLWCQISGHGCLS	12.8	17.2	1.4	12.6	
RP6-6-D815-IGFR-4-A2	SFYSGLACLLSGPAQPNRGKCERSRGDLP	12.7	17.1	1.8	9.4	
RP6-6-D815-IGFR-4-B11	SFESCLAWLLTSRPLSDSVSWDRCRGRYS	12.8	17.1	1.2	14.3	
RP6-6-D815-IGFR-4-D1	SFYSCLASLLSGTPQQTGGRWERGRGCLS	12.5	17.0	1.4	12.4	
RP6-6-D815-IGFR-4-C8	RIGGSGGSWLDQERAWLWCKISGRGCLS	12.7	16.2	1.5	10.6	
RP6-6-D815-IGFR-4-A11	SFYSCLASLLTGAPERDWLVCEMSSRGWGS	11.6	16.0	1.1	15.1	
RP6-6-D815-IGFR-4-E7	RSFYDCLTFVLNGPPQPNGGRWERCR	13.0	16.0	1.5	10.7	
RP6-6-D815-IGFR-4-B9	TFYSCLDSLMSGTPQSLRGRWERCRGYGA	11.5	15.8	6.9	2.3	
RP6-6-D815-IGFR-4-D5	GLSTRGSQLAQERAWLWCEISGRGCVS	12.4	15.8	1.2	13.1	
RP6-6-D815-IGFR-4-C6	SGGSGGSWVDRQRAWLWCEISGRGCLS	13.2	15.7	1.5	10.4	
RP6-6-D815-IGFR-4-E12	WERRCGGSGGSWLDQQRAWLWGEISGRGCVS	10.6	15.6	1.0	16.0	
RP6-6-D815-IGFR-4-B5	TLYACAASRPTGTPQPNLGSWAGSRGGSGGSRLDQERAWLWCEISGLGCMS	12.9	15.6	1.2	12.8	
RP6-6-D815-IGFR-4-B4	SFYSCLASLVTGNPQPNRGCLS	11.6	15.6	1.3	12.3	
RP6-6-D815-IGFR-4-H12	SFYTCLSSLLTGTPPPNRGPWELSRGCVT	12.1	15.5	1.1	14.2	
RP6-6-D815-IGFR-4-B6	TFYSCLASLLDGTTQPHRGSWERCRGGLS	12.1	15.4	1.4	11.2	
RP6-6-D815-IGFR-4-D7	SFYSCLASLVTGAGKSPGRGCLG	11.7	15.4	1.2	13.2	
RP6-6-D815-IGFR-4-D10	SFYSSMACLLNGTPLPDRGPRERWRACLT	11.2	15.3	1.5	10.6	
RP6-6-D815-IGFR-4-B7	SFYSCLDTLLGGTPQQNRSPWDRCRGGVS	11.8	15.3	5.9	2.6	
RP6-6-D815-IGFR-4-E10	HSRGGSGGSWMEKERAWLWCEISGCGGSS	14.2	15.2	1.6	9.7	
RP6-6-D815-IGFR-4-C3	SFYSCLDTLLTGASWKRCRGGLS	12.0	15.0	1.3	11.2	
RP6-6-D815-IGFR-4-F10	TFYSCLDSLLTGTPEPNRGGWERCRGGQY	12.6	14.9	1.4	10.4	
RP6-6-D815-IGFR-4-E6	SFYSCLDTLLTGASWKRCRGGLS	11.8	14.9	1.4	10.5	
RP6-6-D815-IGFR-4-F7	TFYSCLASLVTGAPGPKRGEWERCRGGLP	11.1	14.7	1.5	9.5	
RP6-6-D815-IGFR-4-A5	NQTVVDGYVVVGGSGGSWLDQERAWLWCEISGRDCPT	11.5	14.6	1.2	12.7	
RP6-6-D815-IGFR-4-C10	SFYSCLDTLLTGASWKRCRGGLS	11.2	14.5	1.5	9.5	
RP6-6-D815-IGFR-4-A7	SFYSCLASLLTGTPQTNRGAWDRCRGGQS	11.1	14.5	1.0	14.5	

FIG. 60A

CLONE#	Sequence	Etag	IGFR	LDH	IGFR/LDH	SEQ ID NO:
RP6-6-D815-IGFR-4-F9	DFYSGLACILNGSPEEKHGPWERCRGGLL	12.0	14.4	1.3	11.1	
RP6-6-D815-IGFR-4-B8	SFYSCLDTLLTGASWKRCRGGLS	12.2	14.3	1.4	10.2	
RP6-6-D815-IGFR-4-F12	TFYSCLASLLTDIPEQNRGPRDRCRGGLA	10.1	14.2	0.9	15.7	
RP6-6-D815-IGFR-4-A10	TFYSCLASLLSGPPPPNLDPWDRCRRGPS	10.4	14.2	1.3	10.9	
RP6-6-D815-IGFR-4-E3	SFQSCLASLLTGTTLPNGGTWERCRGWPS	10.9	14.2	1.2	11.9	
RP6-6-D815-IGFR-4-C4	SFYSCLDTLLTGASWKRCRGGLS	11.3	14.1	1.3	11.0	
RP6-6-D815-IGFR-4-C7	TSSLLPNRGAQERIRGGSGGSWLAQERAWLWCEITGRGCLS	12.8	14.0	1.4	10.3	
RP6-6-D815-IGFR-4-C5	TFYSCLASLLNGTPHPNRGPWEISGRGCLS	10.5	14.0	1.7	8.0	
RP6-6-D815-IGFR-4-A12	SFHSCLASLVNGTWRPNRGVCEISGRGGVQ	10.0	14.0	0.9	15.2	
RP6-6-D815-IGFR-4-C11	SFYDCLASLLSVTSLPNRVPQDRCRGFLS	11.3	13.7	1.4	10.1	
RP6-6-D815-IGFR-4-F11	TFYSCLDQLETGTPNANRGAWERCRGGLF	11.2	13.6	0.9	15.9	
RP6-6-D815-IGFR-4-F6	GSVRGNVGSGGSWLDRQRAWLWCEISGRGCPS	10.7	13.5	1.4	10.0	
RP6-6-D815-IGFR-4-C2	VRPDRGTCERCRGGSGGSWLDQERAWLWCEISGRGCLP	10.8	13.4	1.3	10.0	
RP6-6-D815-IGFR-4-D6	SVSKWLERERARLWCEISGLGCLS	11.7	13.3	1.1	11.9	
RP6-6-D815-IGFR-4-E1	SFYSSMACLLNGTPLPDRGPRERWRACLT	11.7	13.3	1.7	8.1	
RP6-6-D815-IGFR-4-A9	TVGNVGGSLRDQVRSWLWCEISGRGCMS	12.0	13.1	1.3	9.9	
RP6-6-D815-IGFR-4-G11	NFQSCLASLVTGTALPNRGTWERCRGFPA	11.1	13.1	1.0	13.2	
RP6-6-D815-IGFR-4-D2	TFYSCLASLLSGPPKPNRDPQESCRGWLS	9.2	13.0	1.3	10.3	
RP6-6-D815-IGFR-4-A3	SFYSCLDTLLTGASWKRCRGGLS	7.9	13.0	1.2	10.6	
RP6-6-D815-IGFR-4-E9	SFYSCLAALLTGVPQPNVGPWERCRGPRY	11.1	13.0	1.5	8.9	
RP6-6-D815-IGFR-4-E11	SFYSCLSSLLNGNLPRNPGRWEGCRGGLS	11.9	12.9	1.3	10.2	
RP6-6-D815-IGFR-4-A8	NMVGGQRDRGGSGGSWLDKERAWLWCEISGLGCRS	10.5	12.8	1.3	9.9	
RP6-6-D815-IGFR-4-H11	TLSGGSSGGSWLDRERAWLWCEMTGRGCRS	10.7	12.7	1.1	11.2	
RP6-6-D815-IGFR-4-E5	TSYSCLASLLPDSPQPNRGQGERRRGGSGGSWLD.ERAWLWCQISGRGCLS	8.0	12.6	1.2	10.2	
RP6-6-D815-IGFR-4-F5	TFYSCLTSLMSGGTWEHCRGGSGGSWLDQERAQQCRGITGRGCPS	8.9	12.6	1.0	12.8	
RP6-6-D815-IGFR-4-D4	TFYSCLASLLTGTPHLNRGPWERCRDGLS	10.7	12.5	1.1	11.5	
RP6-6-D815-IGFR-4-G8	NSASWIDQERAWLWCEISGRGCLS	9.7	12.3	1.1	11.1	
RP6-6-D815-IGFR-4-G9	TFYSCLDFLLAGTPTPARGPWEHCRGGMS	10.4	12.2	1.5	8.0	
RP6-6-D815-IGFR-4-B1	SFYSCLTSLLTGTPKPNRGCVS	7.5	11.9	1.3	9.5	
RP6-6-D815-IGFR-4-F3	TFYSCLDSLRTSTAQVKPGPWERCRGSMS	9.7	11.8	1.4	8.6	
RP6-6-D815-IGFR-4-G5	SFYSCLASLLPGTALPNRGCRS	9.4	11.3	1.0	11.1	
RP6-6-D815-IGFR-4-B2	GFYDCLASLVTGPPESKRGAWERCRGSGS	11.2	11.2	1.5	7.6	
RP6-6-D815-IGFR-4-A4	TFYDCLASLLTDTGQPSRGRWERCRGGLS	9.7	11.1	1.1	9.7	
RP6-6-D815-IGFR-4-G10	TFYSCLAYLVTGTPKPYRGPGETWRGCLS	10.2	11.0	1.4	7.9	
RP6-6-D815-IGFR-4-D3	SFYSCLSALLAGSPEPNRGPGQRCRGSSL	10.1	11.0	1.1	10.2	

FIG. 60B

CLONE#	Sequence	Etag	IGFR	LDH	IGFR/LDH	SEQ ID NO:
RP6-6-D815-IGFR-4-E2	TFYSCLASLVSGSPQRSGGPWERCRGGQS	8.8	10.9	1.3	8.4	
RP6-6-D815-IGFR-4-H8	PAMEDRWYVGRGGSGGSWLDQERAWLWCEISGRGCLS	8.3	10.9	1.4	7.6	
RP6-6-D815-IGFR-4-E4	TFYSCLDELVTGTPQLTRGPWERCRGWLS	8.1	10.8	1.1	9.7	
RP6-6-D815-IGFR-4-F1	GFYDCLASLVTGPPESKRGAWERCRGSGS	9.0	10.5	1.2	8.5	
RP6-6-D815-IGFR-4-H7	SFSSAGSLLANDSQPSAVRGTLRGDSGGSWLDQERAWLWCEISGRGCMS	10.3	10.4	1.8	5.9	
RP6-6-D815-IGFR-4-F4	NFYTGLTPLLSDRTEQNRGRWDGRRGGSGGSWLDQERAWLWCEISGRGCVS	7.9	10.3	1.0	10.6	
RP6-6-D815-IGFR-4-H9	SKPNRDTWERGRGGSGGSWLDQERAWLWCEISGRGCLS	8.0	10.1	1.2	8.5	
RP6-6-D815-IGFR-4-G1	SFYSSMACLLNGTPLPDRGPRERWRACLT	5.7	10.1	1.0	10.5	
RP6-6-D815-IGFR-4-A6	SFYSCLAALLVGNPERERGPWVRCRGGLY	12.8	10.1	1.2	8.7	
RP6-6-D815-IGFR-4-G2	TFYSCLASLRTAPPPNRGPWEGCRGGLS	7.2	9.6	1.0	9.3	
RP6-6-D815-IGFR-4-G3	SFYSCLVGLMNATPDPSRGVWQSCRGGPP	7.4	9.3	0.8	11.6	
RP6-6-D815-IGFR-4-G12	SFYSCLASLVTGTPQACRGPWERGRGYLS	8.9	9.0	1.0	9.3	
RP6-6-D815-IGFR-4-G4	RSWLDKERAWLWCEISGGGCLT	6.7	8.7	0.8	11.6	
RP6-6-D815-IGFR-4-H3	SFYSSMACLLNGTPLPDRGPRERWRACLT	6.4	8.5	0.8	11.0	
RP6-6-D815-IGFR-4-G6	VRGGSRLDLERAWLWCEISGRGCLS	9.6	8.4	1.2	7.1	
RP6-6-D815-IGFR-4-H1	TCFSCLGSQRAGTPERNRGSMGGGGSGGSWLGRERAWLWCEISGRGCLS	5.9	7.6	1.0	7.8	
RP6-6-D815-IGFR-4-H6	SSGERRGGGSGDSWLGEVRAWLWCEISGSGCQS	6.2	7.5	1.3	5.7	
RP6-6-D815-IGFR-4-H2	SFYSCLDTLLTGASWKRCRGGLS	6.6	7.5	0.9	8.8	
RP6-6-D815-IGFR-4-F2	TFYACLANLLSGTPEASRGTWERCRGRES	6.2	7.4	1.1	6.8	
RP6-6-D815-IGFR-4-A1	TFYDCLASLLTQPNRGRGCRL	7.8	6.5	1.5	4.3	
RP6-6-D815-IGFR-4-H4	TFYSCLASLVTGTPQPDRGGWERCRGGRF	5.6	6.0	0.8	7.2	

FIG. 60C

RP6-0-RP9 vs IGFR

Clone #	SEQUENCE	E-Tag	IGFR	LDH	Sp/Irr	SEQ ID NO:
Design	TFYSCLASLLTGTPQPNRGPWERCRGSLDESFYDWFERQLG	--	--	--	--	
RP6-0-RP9-IGFR-B11	TLNPRGPWEGSRGSMDDSFYRWFERQLE	27.6	25.7	1	25.2	
RP6-0-RP9-IGFR-H2	TGAPQPNRGPLDRCRGSLDECFYGWFERQLL	16.6	21.1	1	20.7	
RP6-0-RP9-IGFR-E9	RGNVGGGSLDESFYEWFERQLG	19.8	20.5	0.8	24.2	
RP6-0-RP9-IGFR-C1	TFYSGPVSLLTGTPRTNRSAWERGRGSLDDSFYDWFERQLS	19.6	18.3	0.7	27.7	
RP6-0-RP9-IGFR-D2	TMYSLVGFLPSGVGRPDRGPWERGRGALDESFYSWFERQLD	21.8	17	0.8	22.6	
RP6-0-RP9-IGFR-E1	SLYSCMAALPNGTPQRKPGSWDRSGGHQDDSFYDWFERQLG	20.2	16.4	0.9	18.5	
RP6-0-RP9-IGFR-C5	SLYSGLAFLESGSPEPNRGRWARGRGSRDESFYGWFERQLA	21.3	15.5	0.8	18.5	
RP6-0-RP9-IGFR-C4	TLHAGLDSLVTGPQERNRGPWERSRGALDEAFYGWFERQLG	17.8	15.3	0.9	17.5	
RP6-0-RP9-IGFR-G4	MSSCLASLLTRTPEPNRGRWASGRGPLDGSFYDWFERQLG	21.3	15.2	0.9	16.1	
RP6-0-RP9-IGFR-E7	SLTLSLAAMLPGSQQTGGLALVSGSLDVSFYEWFERQLG	18.5	13.6	0.9	14.9	
RP6-0-RP9-IGFR-F9	TFYSRLVSLPTGTQQPNSGRWVLSSSFVDESFYGWFERQLG	18.2	13.4	0.9	15.2	
RP6-0-RP9-IGFR-B7	TYYSGRGSLDESFYDWFERQLG	20.2	13.1	0.9	15.3	
RP6-0-RP9-IGFR-C3	SFYSCLQSLLIGIPHWNRDPWESCRGSLDEPFFEWFERQLG	16	11.5	0.7	17.2	
RP6-0-RP9-IGFR-H3	SCLSCPASLLTGTPQANRGPRERCRGSLDECFYEWFDRQLG	15.5	11.5	0.8	14	
RP6-0-RP9-IGFR-H10	TIYSWQAAPQTGTPQLHRVPQESCRGSLDECFYDWFERQLG	10.3	11.4	1.1	10.8	
RP6-0-RP9-IGFR-E5	SFQTHLASLVTGTAVTNHGVWERGSGSLDGSFYDWFERQLG	17.2	10.9	0.7	15.1	
RP6-0-RP9-IGFR-A7	NLYSWVPSVRTGTPQPNRGAWECCAGSLDIGFYEWFEHQLG	21.8	10.6	0.8	12.6	
RP6-0-RP9-IGFR-G9	TVYCCVASVLTGTPQASGGAWERGRGSLDEAFYDWFERQLG	11	10	1.1	9.2	
RP6-0-RP9-IGFR-A2	TFDNCLASVLTGSPETRRGPWERSRGSVDEYFYDWFERQVA	15	9.9	0.6	17.2	
RP6-0-RP9-IGFR-E3	TSSGSASLVTDSTQPKRGRQVRCGGSVNECFYAWFERQLG	13.6	9.8	0.9	11.1	
RP6-0-RP9-IGFR-B10	SLYSCGASHHNGTLQPNGGPSDRCAGSLDESFYAWFERQLG	13.8	9.4	0.8	11.4	
RP6-0-RP9-IGFR-H4	SSLSCLAALQNVNAQASRSAGERCLGSRDECFYAWFERQLA	10.8	9.3	1.1	8.5	
RP6-0-RP9-IGFR-D11	TFHGCLASMMTVAPVPNRGPWGRGRGSEDESFYDWFEQQLG	15.3	8	0.8	9.6	
RP6-0-RP9-IGFR-G11	QMRWFSEESFYDWFDRQLS	19.4	7.8	1.1	7.1	
RP6-0-RP9-IGFR-F8	TFHPWLARLLTGTPQQNGGAWDPSRGSLDEQFYGWFERQLG	7	7.4	0.9	8.2	
RP6-0-RP9-IGFR-H8	TSYSRLASMLTGTSQPNRGAWDRGRGSVDEAFYDWFERQLD	7.5	7.3	1.2	6.1	
RP6-0-RP9-IGFR-D5	SFYSCLASLLAGGPETNRGPWGRCRLSPEESIEDWGDSQRG	12.8	7.1	0.9	8.2	
RP6-0-RP9-IGFR-A9	TFQAYLVSFQTGNPQTNRGPLEGGRGSLDQCFYDWFEPQVG	10.8	7	0.9	7.5	
RP6-0-RP9-IGFR-H5	TFYSSLASLLTVAPQASLGPGQRGRGSLDESFYEWFERQLG	10.2	6.7	1.1	5.9	
RP6-0-RP9-IGFR-F7	TFDAQLASLPTRPPHANAGPRERCRGSLDECFYDWFERQLG	7.6	6.6	0.9	7.3	
RP6-0-RP9-IGFR-F10	TFYSSLGSLLTGSPQPNRGSWERGRGPLDEAFYEWFERQLG	4.2	6.1	0.8	7.6	
RP6-0-RP9-IGFR-E10	NSYSFLAAHLIGSPPRNGGSWERGRGSLDESFYEYFERMLG	13.9	6	0.8	7.8	
RP6-0-RP9-IGFR-A3	SLYSCPDYRLTGAPRPNGGQWGRGRGPLDESFYGWFERGLG	10.4	5.6	0.5	10.7	

FIG. 61A

Clone #	SEQUENCE	E-Tag	IGFR	LDH	Sp/Irr	SEQ ID NO:
RP6-0-RP9-IGFR-G3	TVYSWLGVLQTGNPHPNRDPWEGGRGSLDESFYDWFERQLG	9.3	5.6	0.9	6.3	
RP6-0-RP9-IGFR-B4	SFYDCLASLLTGPPQPNRGSQDLCRGSLDETFKDWDEHRLG	4	5.1	0.8	6.3	
RP6-0-RP9-IGFR-C9	SFYSCLASLLTGTPQPNRGSWDRCRGSLDESNQDWFERPRG	8.6	5.1	0.9	5.9	
RP6-0-RP9-IGFR-G6	ESFYDWFERQLG	13.7	5.1	0.9	5.9	
RP6-0-RP9-IGFR-A8	TFQSCLASLLTGAPQPNLGSWKRGSGSMDDSFYDWFERQLS	6.6	4.7	0.7	6.8	
RP6-0-RP9-IGFR-G8	NCYSSLGSLLDGPPHPEPWFLGRQPWFSGRIFLRLVRALAGCGR	7.2	4.6	0.9	5.2	
RP6-0-RP9-IGFR-B12	TIYSGLASLLRGTPEANIGLWERRRGTLDESFYDWFERQLG	10	4.5	0.4	10.1	
RP6-0-RP9-IGFR-B9	TFYSSLASLFLGTPPLNYGPVERRPGSLDESFYDWFDSQLG	7.6	4.4	0.9	4.9	
RP6-0-RP9-IGFR-A4	TFQSCLTSLETAAPQPTRAVWEGSRVSRDESFYDWFERQLS	11.8	4.1	0.7	6.2	
RP6-0-RP9-IGFR-H9	TLQSSLASLVSGSPEPKRVRQERSRGSLDDSFYDWFDRQLG	9.7	3.9	1	3.8	
RP6-0-RP9-IGFR-C8	TSSLSRASQLTGTLQPNRGQSERYRGSLDDSFYEWFERQLG	4.7	3.6	0.8	4.3	
RP6-0-RP9-IGFR-F3	SFYSCVCSLLNGTPQPTRGLWDRCSGFFDESSFDCFAGELG	3	3.4	0.8	4.2	
RP6-0-RP9-IGFR-A1	DVYSWCGLWLTGTPQAQRVSLELYRGSRDESFYDWFERQLG	10.1	3.2	0.5	6.1	
RP6-0-RP9-IGFR-A12	TFYSRQASSWTGALKRNGCPQEGCRGSLDGSFYDWFERQLG	7.3	3.1	0.4	7.5	
RP6-0-RP9-IGFR-E4	SSTSWPGFAWLTGIAQPNLGPWHRLRGSPDEPFYDWFQRQLG	12.5	3	0.8	3.7	
RP6-0-RP9-IGFR-D9	TFHSQLALTAASCSAPGCGPSELYRGSLDESFYTWFERQLG	8.4	2.9	0.9	3.2	
RP6-0-RP9-IGFR-F2	TFNSSLASLLTGTPQATGAPWETWRGQGDELFYDWFERQLG	8.1	2.8	0.9	3.2	
RP6-0-RP9-IGFR-D8	GFYSCLASLVTGTPQPNRGQWVRCRGTLDETVQGKFGGKLG	4	2.7	0.8	3.6	
RP6-0-RP9-IGFR-F5	TSQSGLASLLTGSPKSFGGPGERWGGSLDEAFYDWFERQLG	3.9	2.7	0.8	3.3	
RP6-0-RP9-IGFR-H6	TSSLAWASLLTGSPQLNRGPWESSRGSLDEQFYEWFEHQLG	4.5	2.7	1.2	2.3	
RP6-0-RP9-IGFR-C12	TGHYWRDFELDGTPEPNRGPLERGGGTLDGSFYDWFERQLA	4.9	2.5	0.4	5.7	
RP6-0-RP9-IGFR-B8	TLDSSLVARLNGTPQPNGGPWERIRTSLDESFYEWFERQLG	3.4	2.5	0.8	2.9	
RP6-0-RP9-IGFR-F4	TLYSCRASLVTGNARPNDGPWELCGGSLDESFYDWFERQLG	7.7	2.4	0.9	2.7	
RP6-0-RP9-IGFR-G7	TFYSQAAFSADLYSGQNRWLREHYRGSQDESFYDWFERQLG	4.6	2.2	0.9	2.3	
RP6-0-RP9-IGFR-D12	TFNTWLTPLLNGTGQPNAGRWELCSGSQDECFYGWFERQLG	3.1	2.1	0.4	5	
RP6-0-RP9-IGFR-E8	TLQSGLASLVTGNPKASGGPWGPSRGSLDGNFYDWFERQLS	6.8	2.1	0.8	2.6	
RP6-0-RP9-IGFR-D6	TFDSCMASLIPCTPLPNRGPWERGRGSLDKSFYDWFERQLG	4.7	2	0.8	2.5	
RP6-0-RP9-IGFR-C10	GFYSSLDQMLNGIPQPNRGAWERCRDCLDESFYDWFERQLG	7.4	1.9	0.8	2.3	
RP6-0-RP9-IGFR-E11	SFYSCMASLSTGTLGGAWERCRGSPDETSSESFEREPG	5.1	1.9	0.9	2.2	
RP6-0-RP9-IGFR-E6	TLQPCLASLMAGSPTQDRGPWERVPVAPDESFYGWFERQLS	5	1.8	0.6	2.9	
RP6-0-RP9-IGFR-G10	YFYSGLARLPSGTAQHNRGPWEQRGGSQEGSFYDWFERQMG	5.3	1.7	0.9	1.8	

FIG. 61B

RP6-6-RP9 vs IGFR

Clone #	SEQUENCE	E-Tag	IGFR	LDH	Sp/Irr	SEQ ID NO:
Design	TFYSCLASLLTGTPQPNRGPWERCRGGSGGSGSLDESFYDWFERQLG	--	--	--	--	
RP6-6-RP9-IGFR-C5	VMGGSESMDDSFYDWFERQLG	48.9	22.5	1	23	
RP6-6-RP9-IGFR-D12	TLSSSRASLQAGAPQSNRGRWERCGLVSGGSGSLDDSFYEWFERQLG	46.2	26.4	1	27	
RP6-6-RP9-IGFR-G4	TFYSGVASLMTGTTQPNRGPWERSRGEARGSDSLDASFYDWFERQLV	40.8	21.1	0.8	28	
RP6-6-RP9-IGFR-H8	RSGGPGSVDDTFYEWFERLLG	41.8	16.1	0.8	19	
RP6-6-RP9-IGFR-D10	NFYSYQPSLADWYPGSPTGLHGDVAVALMVGAGSLGDSFYEWFERQLG	46.5	25	1	26	
RP6-6-RP9-IGFR-C11	ISSCLAFSADWLLQGRPVSQWEGRRGGYGGSGSLHDSFYGWFERQLG	15.7	3.3	0.8	4	
RP6-6-RP9-IGFR-A12	GLGSSGSRDESFYDWFERQLG	48.3	30.6	0.8	37	
RP6-6-RP9-IGFR-H10	VLGGSGALDESFYEWFERQVG	48.1	22.7	1	22.1	
RP6-6-RP9-IGFR-G9	WSVGYVGRSGLGGSDSQDESFYDWFERQLG	41.6	22.2	1	22.2	
RP6-6-RP9-IGFR-H3	VVVGSGSLDESFYDWFERQLG	51.3	33.6	1	35.2	
RP6-6-RP9-IGFR-A10	VSGGSGTRDESFYDWFERQLR	35.3	24.9	0.6	38.7	
RP6-6-RP9-IGFR-E12	VRGISGFPEDSFYGWFQRQVD	47.7	35	0.8	41.6	
RP6-6-RP9-IGFR-C6	VPGGSGSLDESFYAWFERQVG	49.2	27.5	1.1	25.5	
RP6-6-RP9-IGFR-E11	VLVAQGSLDESFYAWFERQLG	33.5	14.2	1	14.7	
RP6-6-RP9-IGFR-F1	VLRGSASQDESFYHWFERQVA	47.8	30.4	0.9	32.7	
RP6-6-RP9-IGFR-A2	VLRAPGSLDESFYEWFERQLG	51.3	32.6	1.1	30.7	
RP6-6-RP9-IGFR-A11	VLGSVSLDESLYAWFERQLG	44.8	33.3	0.8	39.9	
RP6-6-RP9-IGFR-F10	VLGRSELLNESFYDWFERQLG	44.8	26.2	1	25.8	
RP6-6-RP9-IGFR-B5	VLGGALDASFYDWFARQLG	48.1	25.3	1	25.8	
RP6-6-RP9-IGFR-B8	VLARSSSLDEAFYDWFERQLG	47.9	29.2	1	29.8	
RP6-6-RP9-IGFR-A1	VGVVVGSLDEQFY*WFERQLG	16.6	10.6	1	10.8	
RP6-6-RP9-IGFR-B3	VACGSDSMDECFYDWFERQLG	33.5	9	1.1	8.2	
RP6-6-RP9-IGFR-F7	TVYPTPASLLDGSPQTNRARWKRDLGGSGGSGALDESFYHWFEDQLR	39.8	12.7	1	12.9	
RP6-6-RP9-IGFR-C9	TSLRLRETVAVGGTRGSGALEESFYDWFERQLG	45.8	16.6	0.9	18.2	
RP6-6-RP9-IGFR-B10	TLYPWPGFYADWYSGAGPVARGNVDGGGSGGSGSLNESFYEWFERQLG	27.1	6.9	0.9	7.8	
RP6-6-RP9-IGFR-C8	TFYTSLAPQLTGPPQPHRGPSARGRVGSGGSGSLDESFYGWFERQLG	21	4.9	1	5.1	
RP6-6-RP9-IGFR-B4	TFYTCLAALVTGNPQPNRGPWERCRAGSGGSGSLDEFYYDGFERQVG	4.6	1.5	1.1	1.4	
RP6-6-RP9-IGFR-E3	TFYTCLAALVTGNPQPNRGPWERCRAGSGGSGSLDEFYYDGFERQVG	2.6	1.4	1.1	1.3	
RP6-6-RP9-IGFR-E2	TFYSCLTSLQTVTPSPNPGSWERSRGGSGDSGSVQESFYDWFERQLG	55.1	16.9	1	17.3	
RP6-6-RP9-IGFR-A3	TFYSCLDALLAGPPEPWDRCRGSPGGLGSLDGSLYDWLEGERG	7	1.8	0.9	2.1	
RP6-6-RP9-IGFR-A5	TFYSCLAALLTAPPQPNGGAWERCRGFGYLDESASDQVQSQLA	11	3.5	0.9	3.7	
RP6-6-RP9-IGFR-E8	TFYS#LPSLLTGTKQPSGGPWERGRGNVGGTGSLNEAFYDWFERQLG	25.2	6.3	1	6.4	
RP6-6-RP9-IGFR-D7	TFYPCQGSPAGLVPLARTVGRGNVARAGSVGSGSLDGSFYDWFERQLG	45.4	13.4	1	13.4	
RP6-6-RP9-IGFR-D8	TFTSGLRYL.PGLSQASVSDRSRGGSGGSGSLDESFYKWFERQLG	16.4	2.9	0.9	3.2	

FIG. 62A

Clone #	SEQUENCE	E-Tag	IGFR	LDH	Sp/Irr	SEQ ID NO:
RP6-6-RP9-IGFR-G5	TFQYCLGAVLSDTPQVNGRSPDRGGGGSGGPGSLDEFFYGWFERQLG	7	2.4	0.8	2.9	
RP6-6-RP9-IGFR-B2	TFQTCMANMRSGTPERNGGSLGGWRASSGGSGSLDESFYDWFDRQLG	35.6	10.6	1	10.3	
RP6-6-RP9-IGFR-B9	TFQSSVASLLTGTLQPHRGPWDRLRGGSGVSGSLDESFYDWFERQLS	4	1.4	1	1.5	
RP6-6-RP9-IGFR-D5	TFNRIQSTLLTGTMQPIRAPKECCRWGARGSGSVGESFYDWFERQLG	1.2	1.2	1	1.2	
RP6-6-RP9-IGFR-G11	SSSFPGFSGRLVLRSGTVVRGDVTLLVPAAVNLDESFYDWFKRQLG	43.9	27.8	1	28.8	
RP6-6-RP9-IGFR-G7	SFYSWLGSSADGQSATEQWPRWDVAGVASRGTGSLDELFYDWFERQLD	22.5	9.1	1.1	8.2	
RP6-6-RP9-IGFR-H5	SFYSGLESLLTGRPQQNRCAWERCRGGSGGQGSLDEGFYDWFERQLG	30.9	21	0.9	24.3	
RP6-6-RP9-IGFR-H7	SFYSALGSLLPGTPQPGRAGGGSGGSGSLDESFYGWFERQLG	41.3	29.1	0.8	37.9	
RP6-6-RP9-IGFR-D11	RSYGSGPLDESFYEWFERQLG	46.1	25.4	0.8	32.4	
RP6-6-RP9-IGFR-F4	PYTSDRGSLADQSSAGRNVGRGRGGWDASGSPHDSFYDWFETQLG	11.5	2.8	1	2.7	
RP6-6-RP9-IGFR-C12	NVH#SLDGLLSGASQPSRGPWGRWLGDSGGSGSLDSSFYDWFERQLG	29.5	6.9	0.9	7.6	
RP6-6-RP9-IGFR-C7	NFGGAGSLDESFYDWFARQLA	14.4	4.1	0.9	4.5	
RP6-6-RP9-IGFR-E5	NFESCGASVMAGTPQNYRRPWERTRGGSSGSGTLDESFYDWFERQRG	43.2	6.7	1	6.7	
RP6-6-RP9-IGFR-E10	MTGPAQRNRVLWEGWPGVSGGSGSMDDSFYGWFNRQLG	40.7	16.6	1	17.2	
RP6-6-RP9-IGFR-F9	MSTLGLAFLRTGTPQPNRGPWGHGGGGSGGSGSLDDSFYDWFERQLG	43.6	20.1	1.1	17.8	
RP6-6-RP9-IGFR-D9	ILSPASLLVPTDTPQAESWIRGDGDRGSSSGAGSQERSFYDWFERQLG	32.7	4.9	0.9	5.3	
RP6-6-RP9-IGFR-B1	HLVALSSLDQSFYAWFERQLG	16.5	7.9	0.9	8.4	
RP6-6-RP9-IGFR-F8	HFYSALGSRGPLERDRGESGGSGSRDESFYDWFERQLG	45.1	24.9	1.1	23.6	
RP6-6-RP9-IGFR-D3	GYRATGSLDGSFYEWFERQLG	50.3	33.9	1	35.3	
RP6-6-RP9-IGFR-G8	GSYSGLATRQTGNQQPNGGQWETGRGGSRGSGSKDESFYDWFERQLG	31.4	7.5	1.1	6.9	
RP6-6-RP9-IGFR-E9	GSVRSGSLDDSFYEWFERQLG	41	26.3	0.8	31.4	
RP6-6-RP9-IGFR-D1	GSVGSASLDKAFYDWFERQLG	32.6	14.3	1	13.8	
RP6-6-RP9-IGFR-B6	GSRGSGSLDTSFYDWFERQVG	52.6	28.1	1	27.6	
RP6-6-RP9-IGFR-C2	GSGVSGSLDESFYDWFERQLR	49.2	33	0.9	36.6	
RP6-6-RP9-IGFR-F3	GSGRSGFQDSAFYEWFERQLG	50.1	29.5	1	29.5	
RP6-6-RP9-IGFR-C10	GSGRGGSLDESFYDWFERQVS	45.1	30.5	0.9	34.1	
RP6-6-RP9-IGFR-E6	GSGHQDFLEESFYDWFERQLA	26.7	8.1	0.7	11.8	
RP6-6-RP9-IGFR-F11	GSGGYASRDESFYEWFERQLA	52.4	36.5	0.9	40.7	
RP6-6-RP9-IGFR-F6	GSGGSGTLDEYFYDWFERQLR	0.9	12.2	1	12.4	
RP6-6-RP9-IGFR-F5	GSGGSGSLNASFYEWFERQLS	37.2	13.2	1.1	12.3	
RP6-6-RP9-IGFR-B7	GSGGRGSLDESFYDWFRSQLG	50.1	31.9	1	33.2	
RP6-6-RP9-IGFR-G3	GRGGTGSLDASFYEWFERQLG	55	36.1	1	37.9	
RP6-6-RP9-IGFR-D2	GLGGSGSKDESFYEWFERQLS	50.8	36.8	1	38.4	
RP6-6-RP9-IGFR-G1	GGVGSGSRDESFYDWFERQLA$_2$	47.6	35.5	0.9	40.1	
RP6-6-RP9-IGFR-C1	GASGAGSLDKSFYAWFARQLD	29.7	11.6	1	11.6	
RP6-6-RP9-IGFR-E7	ESFYEWFERQLG	32.2	19	0.6	29.9	

FIG. 62B

Clone #	SEQUENCE	E-Tag	IGFR	LDH	Sp/Irr	SEQ ID NO:
RP6-6-RP9-IGFR-A6	DSFYEWFERQLG	38.7	18.4	1	18.4	
RP6-6-RP9-IGFR-H4	DLVGLGSLNESFYDWFERQLG	46.4	30.1	0.9	32.9	
RP6-6-RP9-IGFR-B11	DIYLAGYPADRYYATEPLGRWERSRRGSGGSSSLDESFYNWFERQLG	42.9	17.9	0.8	22.5	
RP6-6-RP9-IGFR-B12	DHLLPCAASSAALGTP*PSRGSVGALPGAVLDGFGSIDESFYEWFESQLR	26.5	6.9	0.8	8.3	
RP6-6-RP9-IGFR-D6	DFSTLAWESLLDWSSVVQPWVRGERGRGGSGVTGSLDQSFYDWFERQLG	28.6	6.3	0.7	8.5	

FIG. 62C

D815-RP6 vs IGFR

CLONE	Sequence	Etag	IGFR	LDH	IGFR/LDH SEQ ID NO:
Design	WLDQERAWLWCEISGRGCLSTFYSCLASLLTGTPQPNRGPWERCR	--	--	--	--
D815-RP6-IGFR-4-D10$_2$	RSTFYSCLDTLLTGAPRGNRGTWECWG	12.3	14.1	1.0	13.6
D815-RP6-IGFR-4-G10$_2$	GQFSGGGTVSSFYSCLGSLLTGSPKSRRGPWESCR	13.9	14.0	1.2	11.4
D815-RP6-IGFR-4-E11	WPAREISAGGRQSTFYACLSSLLSGSLQGTGAPWERCR	12.5	13.8	1.0	13.8
D815-RP6-IGFR-4-C10	GGFYSCLDSLLTGSPQPKRGPWERCR	13.3	13.2	1.0	13.2
D815-RP6-IGFR-4-C5	WADRERDSLWRANSSRGYRSTFYSCLSDLLSGTPQQKGGPWEHCR	11.4	12.5	1.0	12.5
D815-RP6-IGFR-4-G11	SSFYSCLTSLVTGSRQWNGGPWDRCRA	11.9	12.1	0.9	13.3
D815-RP6-IGFR-4-D3$_2$	QMGSFYSCLSNLLSGTPQPDRGPWAGCR	9.9	12.0	0.9	13.5
D815-RP6-IGFR-4-B4	SSFYSCLASLLTDNPRAGRGSWERCR	11.0	11.9	1.0	11.9
D815-RP6-IGFR-4-B9	FGGSGGSTFYSCLASLLTGAPQPNRGAWERCR	11.2	11.9	0.9	13.0
D815-RP6-IGFR-4-E4	AWDQERGWPRSTFYSCMASLLTGNPQGNRGPWEGCR	12.4	11.7	1.0	11.9
D815-RP6-IGFR-4-C8	GMVKVGAWLRDESSGRGSRSPFYSWLDCLVTVTPQADGGAWERCR	11.0	11.6	0.9	13.5
D815-RP6-IGFR-4-C12	NSGRGSVSTFYSCLDALSSGTRQANGGLWERCR	12.3	11.5	1.0	11.3
D815-RP6-IGFR-4-C4	SSFYSCLASLLTDNPRAGRGSWERCR	10.8	11.4	1.0	11.9
D815-RP6-IGFR-4-B10	QLDKEGAWPRCDNSGRGCRSTFYSCLWSLVTGTEQLKRGPWELCR	9.4	11.2	1.0	11.0
D815-RP6-IGFR-4-D5$_3$	FRGGQSTFHSCLFSLLSSTPRDNGRPWGRCR	10.2	11.0	0.9	12.7
D815-RP6-IGFR-4-D7	WQGQQGGGKALGAGDVNFYSCLDSLLTGTPEANRGTWEGCR	9.8	10.7	0.9	11.6
D815-RP6-IGFR-4-C6	WAGFYGCLASLVTGTPRPQGGPGECRH	9.7	10.5	0.8	13.3
D815-RP6-IGFR-4-B3	QGWVTSFYSCLGSLLAGTSQAKGGPWQRCR	11.5	10.5	0.9	11.6
D815-RP6-IGFR-4-E12	LVDRNFYCGLASLLTGTPRQNGSPQGRCL	11.0	10.3	1.1	9.7
D815-RP6-IGFR-4-E8	QIGSETFYSCLTSLLRGTPQPNRGPLERCR	10.0	10.3	0.9	11.5
D815-RP6-IGFR-4-E3	DTFYSCLASLVTGTRAENRGPGVSCR	10.6	10.2	0.9	11.4
D815-RP6-IGFR-4-C7	WMEQESAGLRTEISGRRSQSSFYSCMDSLLSGNAESDGRQWERCR	11.4	9.9	0.9	11.0
D815-RP6-IGFR-4-E2	SSFYSCLTSLVTGSRQWNGGPWDRCR	8.6	9.9	0.9	10.5
D815-RP6-IGFR-4-F12	WAELERARLWREMSGGGGLTGFYSCLASLVTGTPLANRGSWERCR	11.2	9.8	1.2	8.5
D815-RP6-IGFR-4-E10	STFYSCLASLLPGHREPSGGPWDRCR	11.1	9.8	0.9	10.5
D815-RP6-IGFR-4-D11	CTFYSCLASLLTGTPEQNRGWWECCR	12.4	9.8	1.0	9.8
D815-RP6-IGFR-4-A6	VAHRSTFYSCLGALLTGFPRENRDSWESCR	8.4	9.5	1.1	9.1
D815-RP6-IGFR-4-B6	WSGLGSGGSTFYSCLGSLLTGTPRRNLDWWEGCR	8.8	9.4	0.8	12.2
D815-RP6-IGFR-4-B12	STCVGCEISGRSQLRTFYSCLDALVTGSAQPNRRPWERCR	10.3	9.4	0.9	10.2
D815-RP6-IGFR-4-A4	WLGQERAPATFYSCLGALLTGPPQPNRGPWDGCR	5.9	9.2	1.1	8.4
D815-RP6-IGFR-4-C2	RLDQDPVSGGERSGRSGLSSFYACLGSLVTGAAQRNRGPWERCR	10.3	9.2	1.0	9.6
D815-RP6-IGFR-4-G9	QLDRDFYFCLDALLTGTAQPNGGPWARCR	10.7	9.2	1.0	9.4

FIG. 63A

CLONE	Sequence	Etag	IGFR	LDH	IGFR/LDH	SEQ ID NO:
D815-RP6-IGFR-4-E9	WPDLERAGPRGEI.GRGGRSTFYSCLASLVAGTAQPNRGPWERCR	10.8	8.7	0.9	9.3	
D815-RP6-IGFR-4-E5$_2$	WMDLSVVASRSTFYSCLGSLLTGAPLQNRGLWERCR	8.3	8.7	0.9	9.3	
D815-RP6-IGFR-4-D6	VGGTFYSCLASLLTGSPPPNRGAWERCR	9.0	8.5	0.7	11.5	
D815-RP6-IGFR-4-G4	SSFYSCLTSLVTGSRQWNGGPWDRCR	7.8	8.5	0.8	11.0	
D815-RP6-IGFR-4-G3	SNFYSCLDSLLTGFPQPNGSQWEGCR	8.2	8.5	0.8	10.8	
D815-RP6-IGFR-4-B7	GLDQDRAWLWSGLSPRAGPSTFYSCLASLLTGTPQPNRGPGARCR	8.9	8.5	0.9	9.4	
D815-RP6-IGFR-4-A10	NSAVVGSLSTFYACIASLVKGTQQPSPWERCR	9.3	8.4	0.9	9.8	
D815-RP6-IGFR-4-G7	TLDPSFYSCLASLMTGTLPPNHGLSERCR	9.1	8.0	0.9	9.3	
D815-RP6-IGFR-4-C11	SQDQVGASLGSAISGRGGLSNFYSCLTSLATDTTRPSRRPWERCR	12.2	8.0	1.0	8.3	
D815-RP6-IGFR-4-B1	RLGQERAWFWGESSGRGGLGSFYGCLADLVRGNPQANSGPQGRCR	6.6	8.0	1.0	8.0	
D815-RP6-IGFR-4-E1	WRPSFNSCLDSLLTGRGRPSGGLWERCR	8.0	7.9	1.0	7.8	
D815-RP6-IGFR-4-A11	TFYSCLESLLTGSPQPNRGAWERCR	8.3	7.8	0.6	13.4	
D815-RP6-IGFR-4-F2	GLGQEGAQLSCSMSDTFYSGLCSLLTGTRQQNSGTWERFH	10.1	7.8	1.1	7.2	
D815-RP6-IGFR-4-G2	QVDRERAWPGCDLSGRGSLDAFYSGLASLLTATRQRNRGPWELCG	7.2	7.7	1.0	7.6	
D815-RP6-IGFR-4-C1	SGFYSCLASLMAGTTQRNRYQLEGCR	7.2	7.6	1.0	7.5	
D815-RP6-IGFR-4-A2	LPVSFYSCLASLQTGAPQPSRGPWERCR	7.2	7.4	1.0	7.1	
D815-RP6-IGFR-4-A5	GGSTFYSCLTSLLAGTPRPRGDLWGSCV	9.3	7.4	1.1	7.0	
D815-RP6-IGFR-4-B2	WGGSRSTFYSCLDSLLLRTAPQNGGQWDGCF	10.0	6.7	0.9	7.3	
D815-RP6-IGFR-4-G6	WTEETRGMVRN.GRLATFYSCLDSLLTVTSQPDRGPWGRCR	10.3	6.5	0.9	7.0	
D815-RP6-IGFR-4-A8	SAFYSCLASLLTGTPRPNGGPWDSCR	9.8	6.4	1.0	6.6	
D815-RP6-IGFR-4-A1	WAGPGTFSFYSCLASLLTSTSQPKRGLWERCR	5.2	5.3	1.0	5.2	
D815-RP6-IGFR-4-H2	WMDQGRLTTFESCINSLLTGIGQPNRGPWERCR	6.3	4.2	0.8	5.0	
D815-RP6-IGFR-4-G1	GQDRERAQLWCEISSGGTFYSGLASLLSGTREPTRGPRTRCR	5.6	4.1	0.9	4.7	
D815-RP6-IGFR-4-H11	VTFYSCLASLGAGTPQPNRGPWDRCR	8.2	3.4	1.0	3.4	
D815-RP6-IGFR-4-D9	QLDQGRAWLWCEISGSGCLPTFESCLATLLTGTPERNRGPWDSCR	4.8	2.0	1.0	2.0	

FIG. 63B

D815-6-RP6 VS IGFR

Clone	Sequence	Etag	IGFR	LDH	IGFR/LDH	SEQ ID NO:
Design	WLDQERAWLWCEISGRGCLSGGSGGSTFYSCLASLLTGTPQPNRGPWERCR	--	--	--	--	
D815-6-RP6-IGFR-4-H10	SVEPENKRGGGLKSFYSCLASLLSGTPEQDRGPWEGCR	14.9	17.7	1.3	14.1	
D815-6-RP6-IGFR-4-D11	SSFYSCLASLLTDTPQPRRGKWERCR	12.7	17.4	1.4	12.8	
D815-6-RP6-IGFR-4-D9	QNLSPWQLSGGSGGSHFYSCLASLLTGTPQPNRGTGDRCR	13.4	17.3	1.6	10.7	
D815-6-RP6-IGFR-4-D1	SLDQGRAGRSTFYSCLATLLMGTPQPNGGPWKDCR	12.5	17.0	1.4	12.4	
D815-6-RP6-IGFR-4-E8$_2$	GLGQERGGSTFYACLASLVTGSGQANHGLGERCR	12.5	16.1	1.4	11.3	
D815-6-RP6-IGFR-4-B9$_2$	SLLSGGSTFYSCLASLRTGGPQANRGPWERCR	11.5	15.8	6.9	2.3	
D815-6-RP6-IGFR-4-D5	RLDRERPWVSLDSSVRGWLDGGSGGSTFYSCLGSLLADTPERNRGSWKRCR	12.4	15.8	1.2	13.1	
D815-6-RP6-IGFR-4-C6	WDRTRSGGSSFYSCLGSLLGGFTQPDRGQWEGCR	13.2	15.7	1.5	10.4	
D815-6-RP6-IGFR-4-B4	WQDPDRAGLWRVSYGRG.VFGGSGGSSFYSCLASLVSGTRQPNRGPWERCR	11.6	15.6	1.3	12.3	
D815-6-RP6-IGFR-4-E12$_3$	TGGSGGSTFYSCLVALLGGPPQQKGGAWGRCR	10.6	15.6	1.0	16.0	
D815-6-RP6-IGFR-4-B5	QARPGSGGSSFYSCLAALLTGDAQPNRGSRVRCR	12.9	15.6	1.2	12.8	
D815-6-RP6-IGFR-4-H12	RGSTFYSCLGSLLTGTPHRDRGPWERCR	12.1	15.5	1.1	14.2	
D815-6-RP6-IGFR-4-D7	LVEQERAQLGCEIAGRGCLSGGSGGSTFYSCLASLINGTLQSNRGATEGCR	11.7	15.4	1.2	13.2	
D815-6-RP6-IGFR-4-B6	QVDQDSSGSTFYSCLASLLTGATQPSGGPWERCR	12.1	15.4	1.4	11.2	
D815-6-RP6-IGFR-4-E10	WAAKEGASFYSCLASLLNGTPLPTRGPWERCR	14.2	15.2	1.6	9.7	
D815-6-RP6-IGFR-4-D8	RVGEISSRGFLAGGSGGSTFYHCLASLLTWTPEANGDPWERCR	12.2	15.1	1.4	11.1	
D815-6-RP6-IGFR-4-C3	WRDKEQAGLWSEISGRGSPSGGSGGSTFYACLAALLSGTQEPNSGKWERCR	12.0	15.0	1.3	11.2	
D815-6-RP6-IGFR-4-E6	WQDEERPWTWREGSGRGRLSGGSGGSTFYSCIAALLHGTPQANRGTWEGCR	11.8	14.9	1.4	10.5	
D815-6-RP6-IGFR-4-F7	WMDQGRAWTWIEISGRGSLSGGSGGSSFYSCLASLMTGAPLPKRGAWEPCR	11.1	14.7	1.5	9.5	
D815-6-RP6-IGFR-4-A5	SVDQERGAISGRGRLSGGSGGSTFYSCLASLLTGPTKPDRGPWEGCR	11.5	14.6	1.2	12.7	
D815-6-RP6-IGFR-4-B8	WLEQESEIARRGWLSGGSGGSTFYSCLGSLLTGSTEQNQGPSVACR	12.2	14.3	1.4	10.2	
D815-6-RP6-IGFR-4-F12	WRDEERGWAGREISGRGGLSGGSGGSTFYSCLSSLLIGTRQPGRGAWEPCR	10.1	14.2	0.9	15.7	
D815-6-RP6-IGFR-4-E3	GVEQERDWLGGGISGRGGLSGSGGSNFYSCIASLLGGTPHPERGSWERCR	10.9	14.2	1.2	11.9	
D815-6-RP6-IGFR-4-C4	WVNQDRGSGGSSFYSCLASLLNGTAKPYRCR	11.3	14.1	1.3	11.0	
D815-6-RP6-IGFR-4-D12	GLDPQGGSTFYSCLAYLLAGTPQWNRGPGERCR	10.6	14.1	1.1	12.5	
D815-6-RP6-IGFR-4-C5	WLDLEMLGSGGSTFYSCLASLMRGPPQPNRGPWGSCR	10.5	14.0	1.7	8.0	
D815-6-RP6-IGFR-4-C11	RVDQERGSGGSAFYSCLAYLLTGPPQPGRGPLERCT	11.3	13.7	1.4	10.1	
D815-6-RP6-IGFR-4-F6	WMDQERGSLRGLSGGSGGSSFYSCLASLLTGPPQGCRGAGEGSR	10.7	13.5	1.4	10.0	
D815-6-RP6-IGFR-4-C2	SGSGGSTFYSCLASLLTGTSQPGRGPRVRCR	10.8	13.4	1.3	10.0	
D815-6-RP6-IGFR-4-E9	WLDQESAGGSGGSTFYSCLASLLNGTPQPNRGPWERCR	11.1	13.0	1.5	8.9	

FIG. 64A

Clone	Sequence	Etag	IGFR	LDH	IGFR/LDH	SEQ ID NO:
D815-6-RP6-IGFR-4-A3	STFYTCLASLLTGAPAPKRGAWERCV	7.9	13.0	1.2	10.6	
D815-6-RP6-IGFR-4-D2	GSEQDRAGVQSKIVGRGRHSGGSGGSGFYSCLADLLTGTALRNRGLWDRCR	9.2	13.0	1.3	10.3	
D815-6-RP6-IGFR-4-E11	WAGPGTCFGRGCVSGGSGGSTFYSCLASLLPGTPQPNRGPGDGCR	11.9	12.9	1.3	10.2	
D815-6-RP6-IGFR-4-A8	SSFSSCLASLVSGTWKPNRGLWERCR	10.5	12.8	1.3	9.9	
D815-6-RP6-IGFR-4-F5	WGSTFYSCLASLLTGTPQAKRGPWERCR	8.9	12.6	1.0	12.8	
D815-6-RP6-IGFR-4-E5	FRGQERAGVRGETSGLGSLSGGSGGSTFYSCLASLLTGIPQPNRGAWVRCR	8.0	12.6	1.2	10.2	
D815-6-RP6-IGFR-4-D4	QVDQERARLGGENSGRGFAPGGSGGSTFYSCLDSLLTGTPMSNRGAWERCR	10.7	12.5	1.1	11.5	
D815-6-RP6-IGFR-4-B1	GAKAVVVGRSGGSGGSTFYSCLASLLTGTAPDNRGPWEHCR	7.5	11.9	1.3	9.5	
D815-6-RP6-IGFR-4-A4	QLAHERAWMPGGSGGSTFYSCLASLLTVTRQLNGGRWERCR	9.7	11.1	1.1	9.7	
D815-6-RP6-IGFR-4-H8	SLDQERAWLRGEISGRGSGSGGSGGSSFYSCLASLLSGTWQPGGGSGDRCR	8.3	10.9	1.4	7.6	
D815-6-RP6-IGFR-4-E2	QNDQSTFYSCLASLMTSTPRPERRPWECRG	8.8	10.9	1.3	8.4	
D815-6-RP6-IGFR-4-F1	WMVQEFAWLGGDISGRGSVSGGSGGSTFYTCLAALVSGTP.TNRGQWERCR	9.0	10.5	1.2	8.5	
D815-6-RP6-IGFR-4-H7	QLEKESGGSGGSTFYCWLRSLVTGTPHSDGSTWERGC	10.3	10.4	1.8	5.9	
D815-6-RP6-IGFR-4-G1	RLEQEGVGCGSKSVGGCLAGGSGGSTFYSCLAALLTGTPEGNRGAWDRCS	5.7	10.1	1.0	10.5	
D815-6-RP6-IGFR-4-A6	QLDPDGSTFYSCLSSLLSSTGQTNRGPWDRCR	12.8	10.1	1.2	8.7	
D815-6-RP6-IGFR-4-G12$_2$	SGGSTFYSCLGVLLTGTPQANRGGWEHCR	8.9	9.0	1.0	9.3	
D815-6-RP6-IGFR-4-C1	RAQQERGGLSSEFSRGGLLSGGSGGSNFYSCLATLLTGTPLPKRGASEGCR	9.4	8.3	1.4	6.0	
D815-6-RP6-IGFR-4-H2	WGSGGSTFYSCVASLLAGGPQPNRGVWERCR	6.6	7.5	0.9	8.8	
D815-6-RP6-IGFR-4-A1	QLGGSSFYSCLASLLTGTPQAYRGSWERCR	7.8	6.5	1.5	4.3	
D815-6-RP6-IGFR-4-H4	WLGQERRWQGGEIVGRGGLSGGSGGSGFYSCLSSLLGRSPERTRGPWELCR	5.6	6.0	0.8	7.2	

FIG. 64B

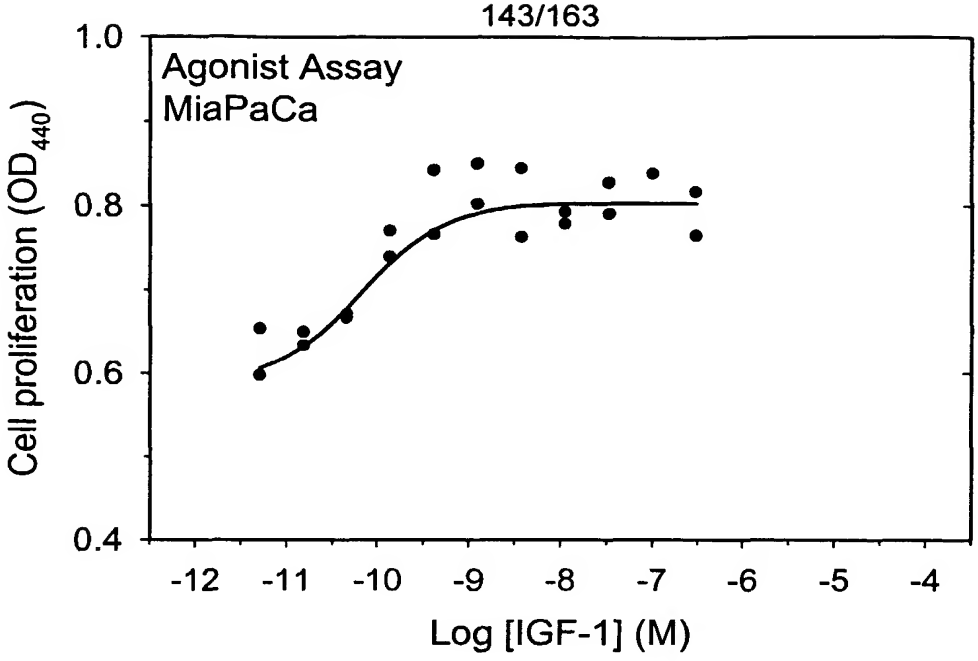


FIG. 65A

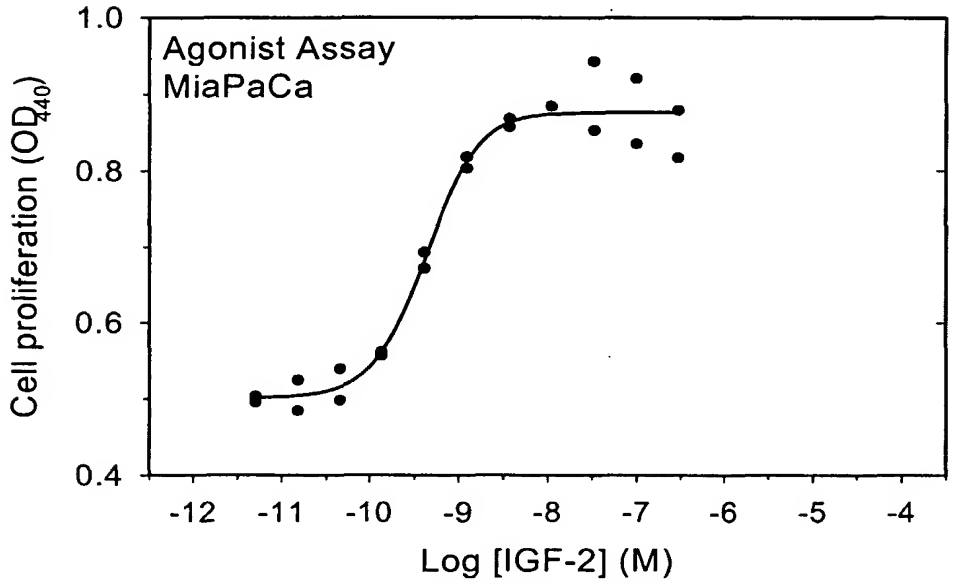


FIG. 65B

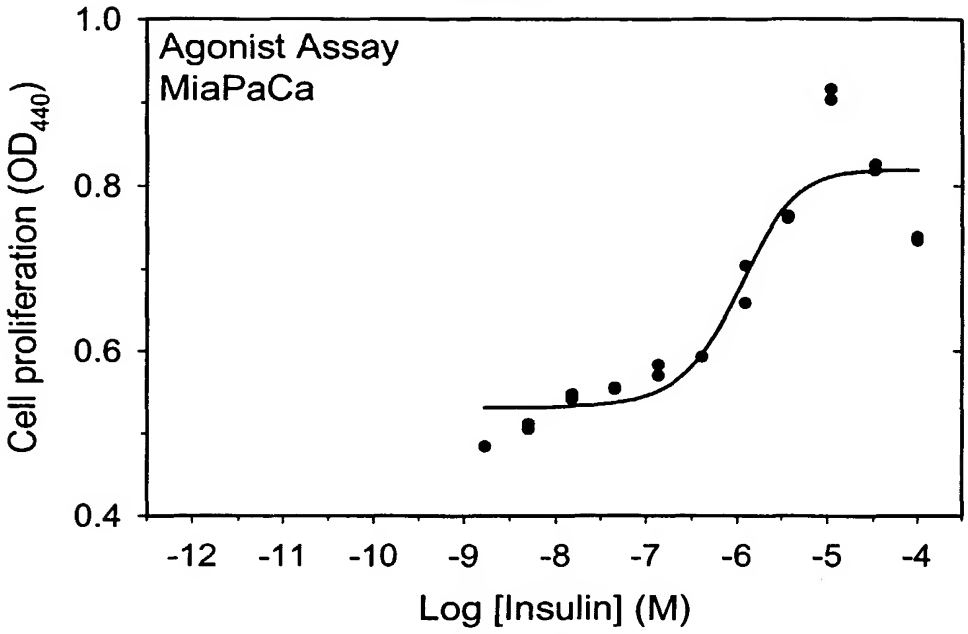


FIG. 65C

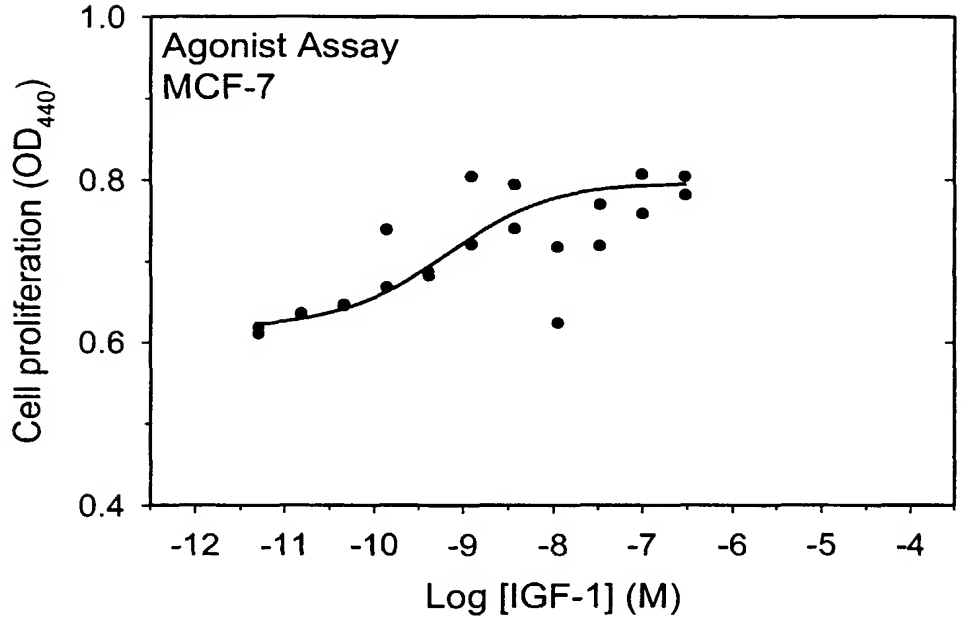


FIG. 65D

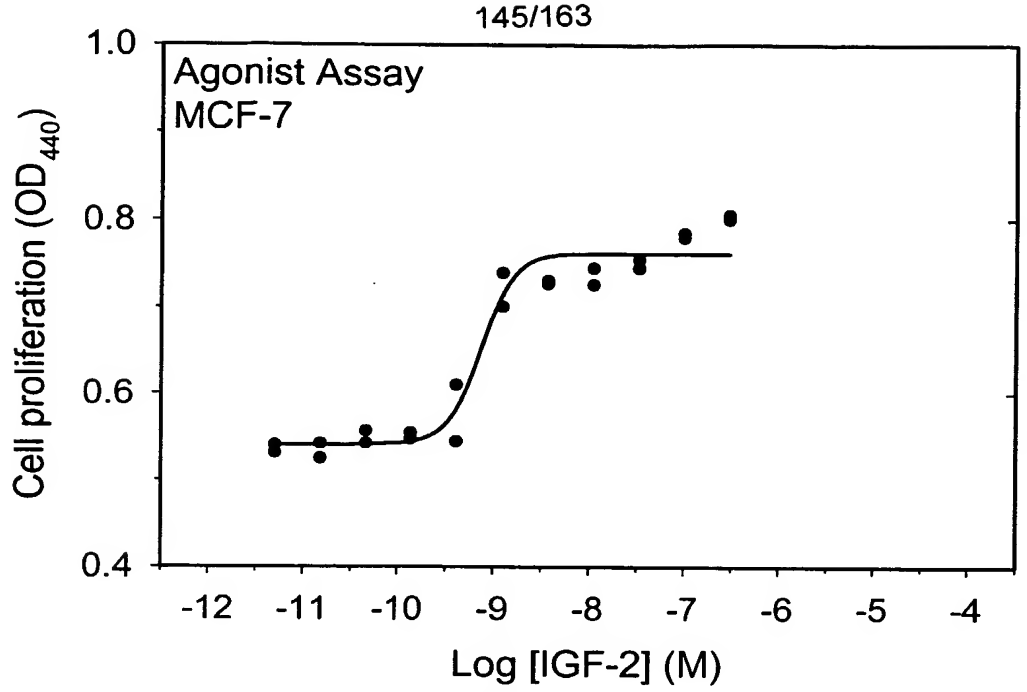


FIG. 65E

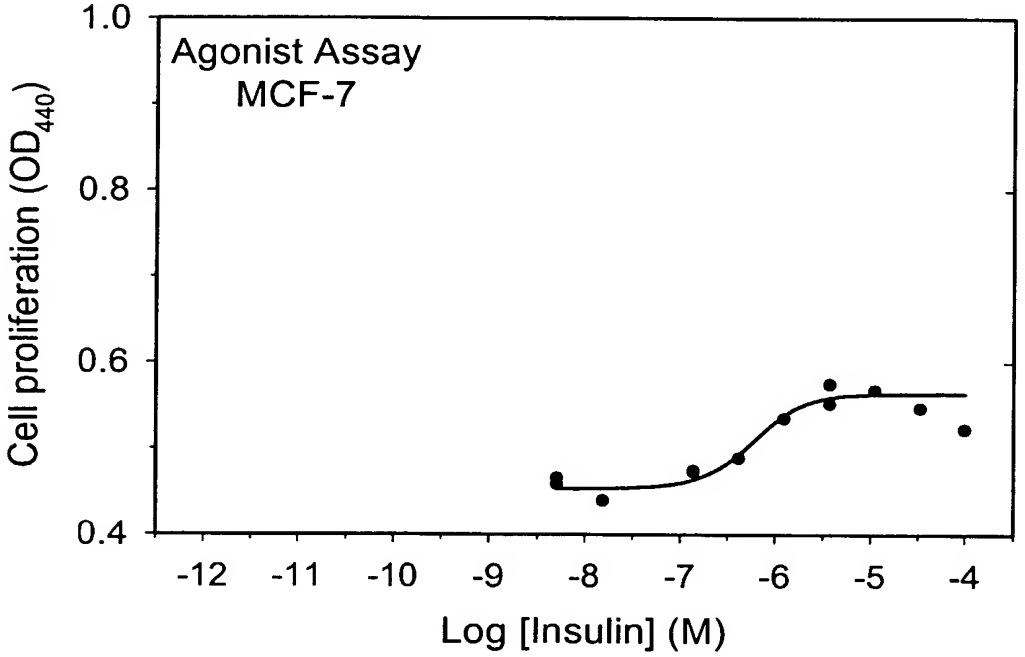


FIG. 65F

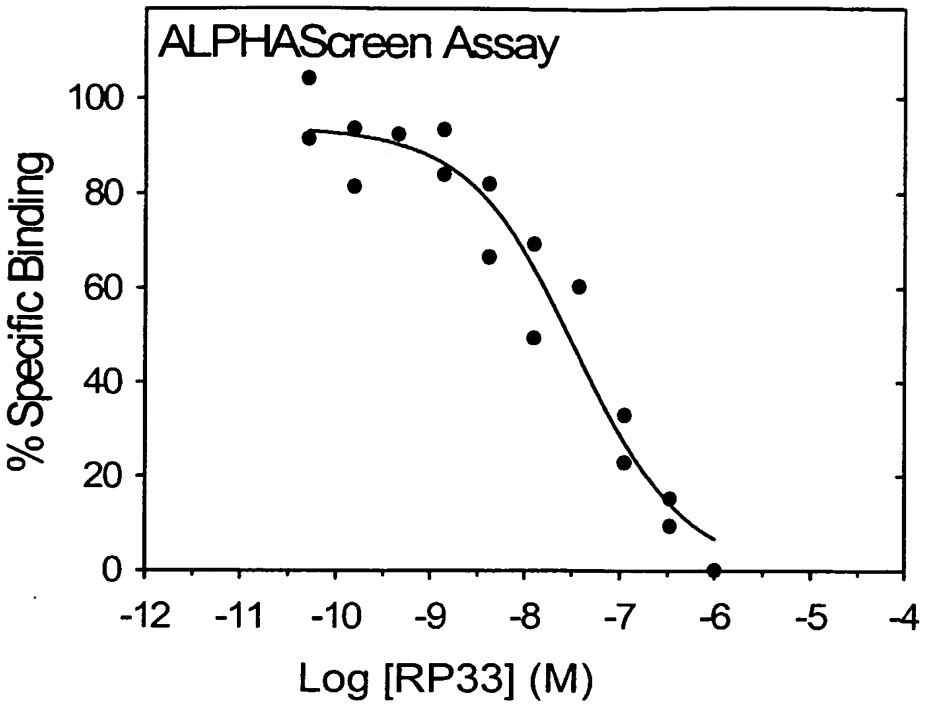


FIG. 66A

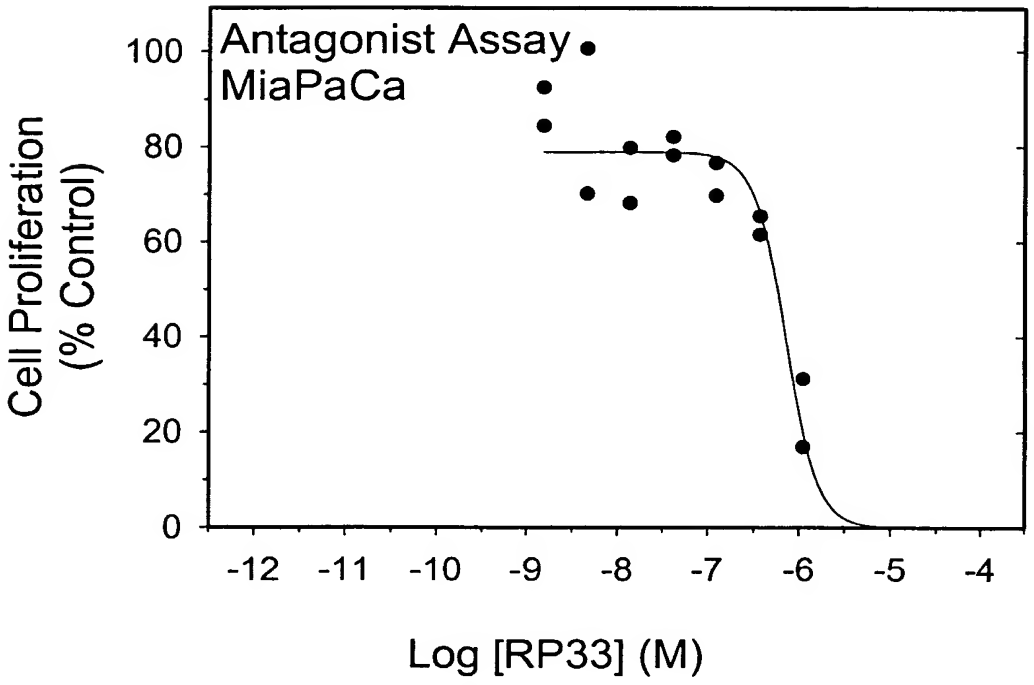


FIG. 66B

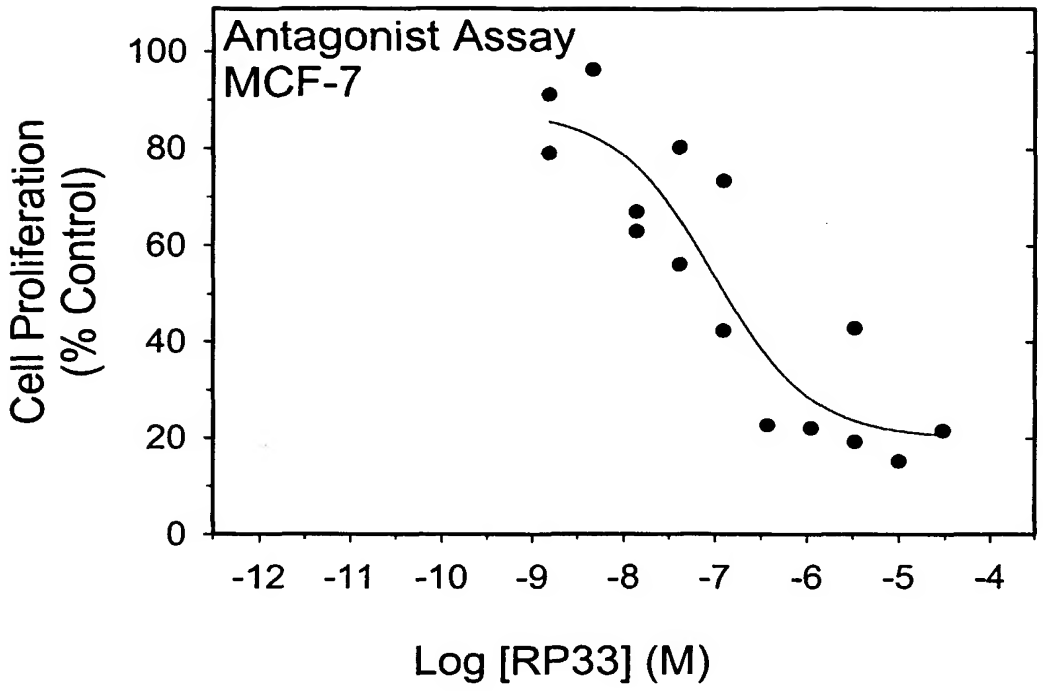

Antagonist Assay
MCF-7
Cell Proliferation
(% Control)
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
-4
Log [RP33] (M)

FIG. 66C

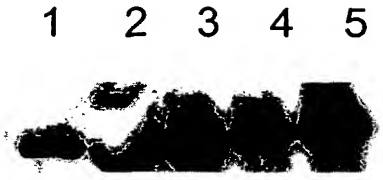


FIG. 67A

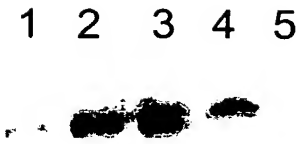


FIG. 67B

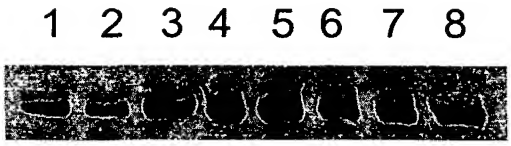


FIG. 68A

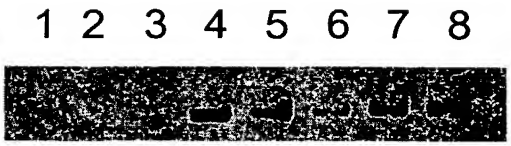


FIG. 68B

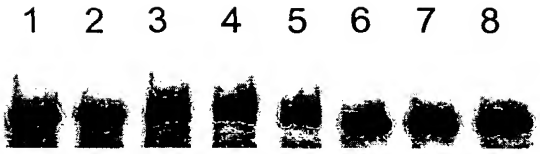


FIG. 69A

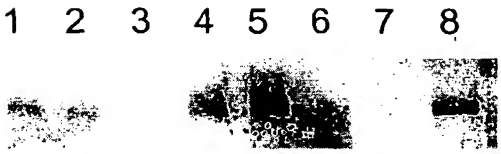


FIG. 69B

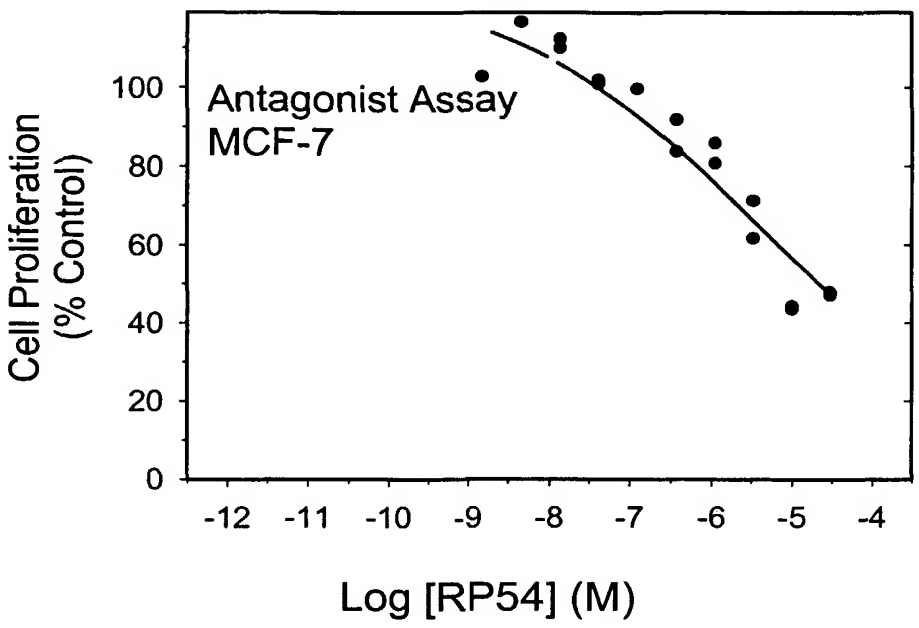


FIG. 70A

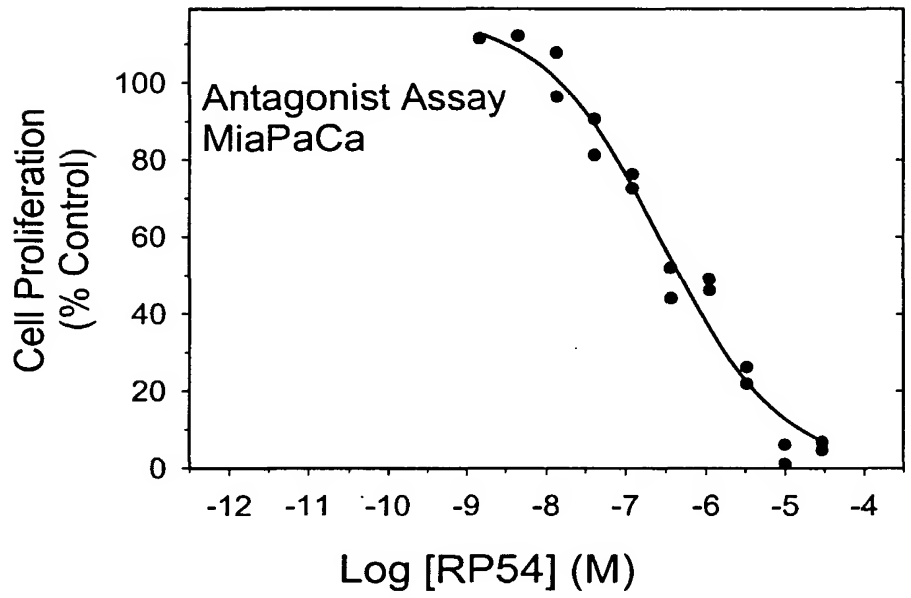


FIG. 70B

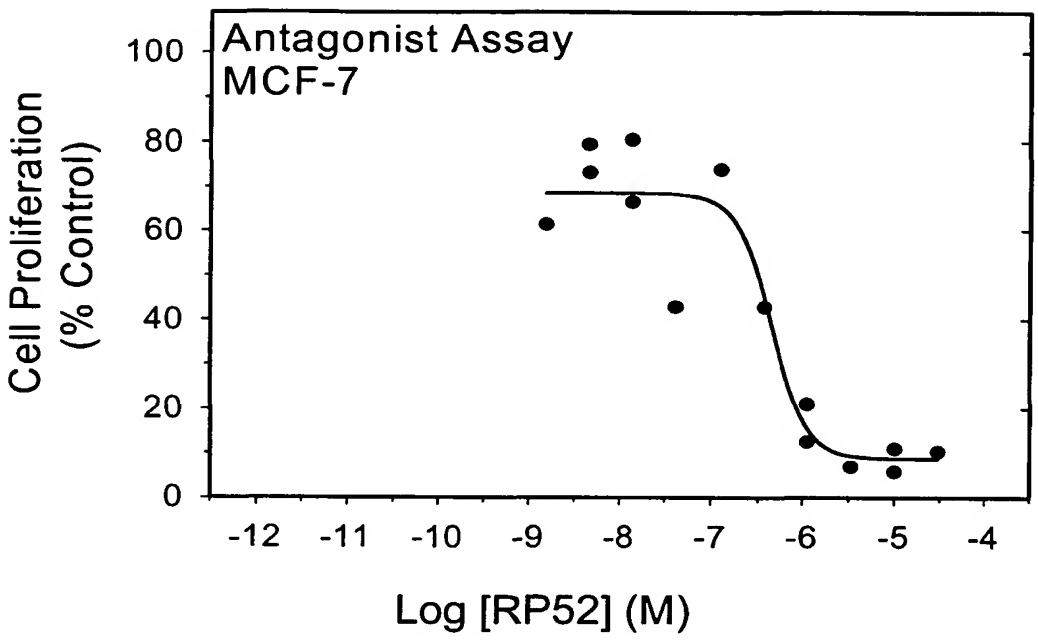

Antagonist Assay
MCF-7
Cell Proliferation
(% Control)
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
-4
Log [RP52] (M)

FIG. 70C

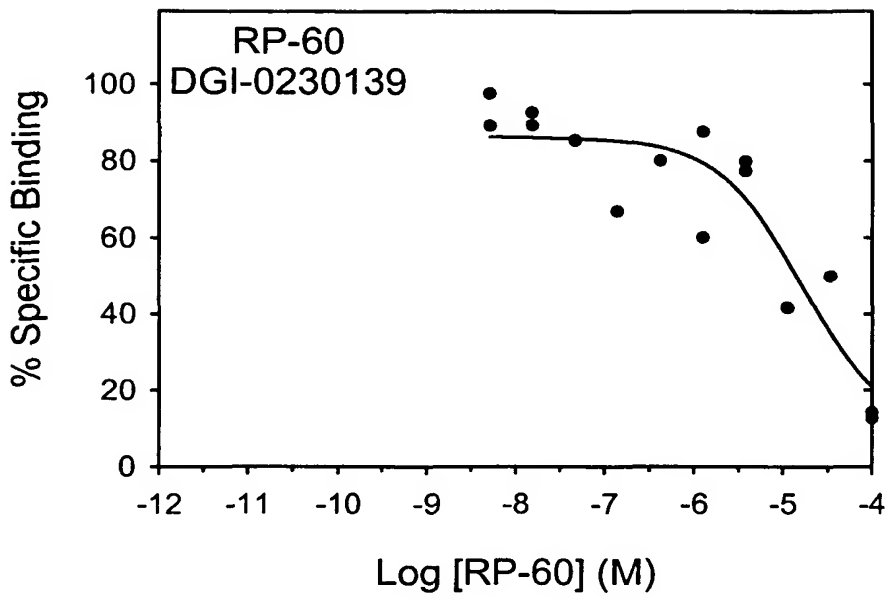


FIG. 71A

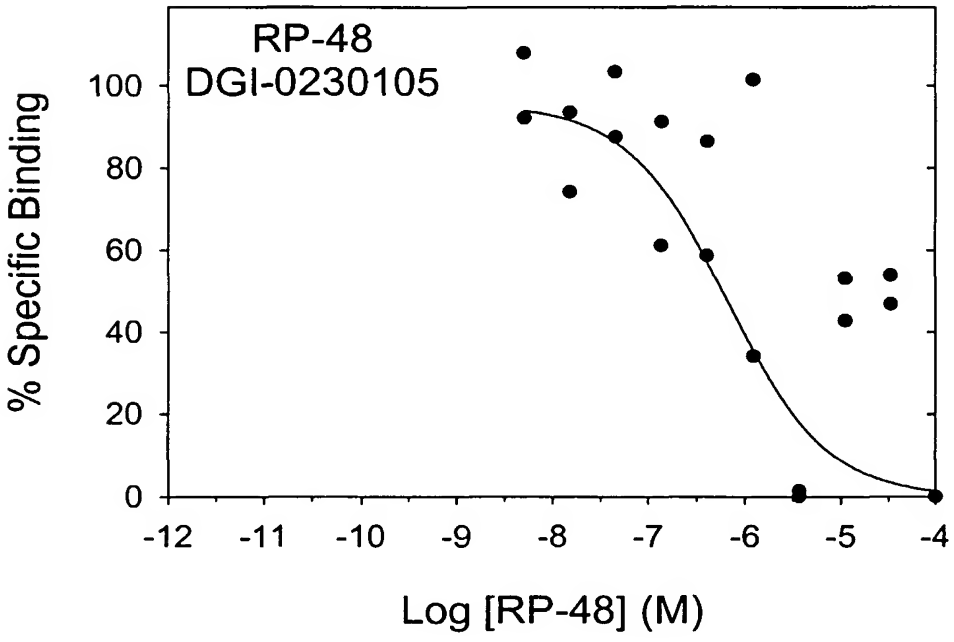


FIG. 71B

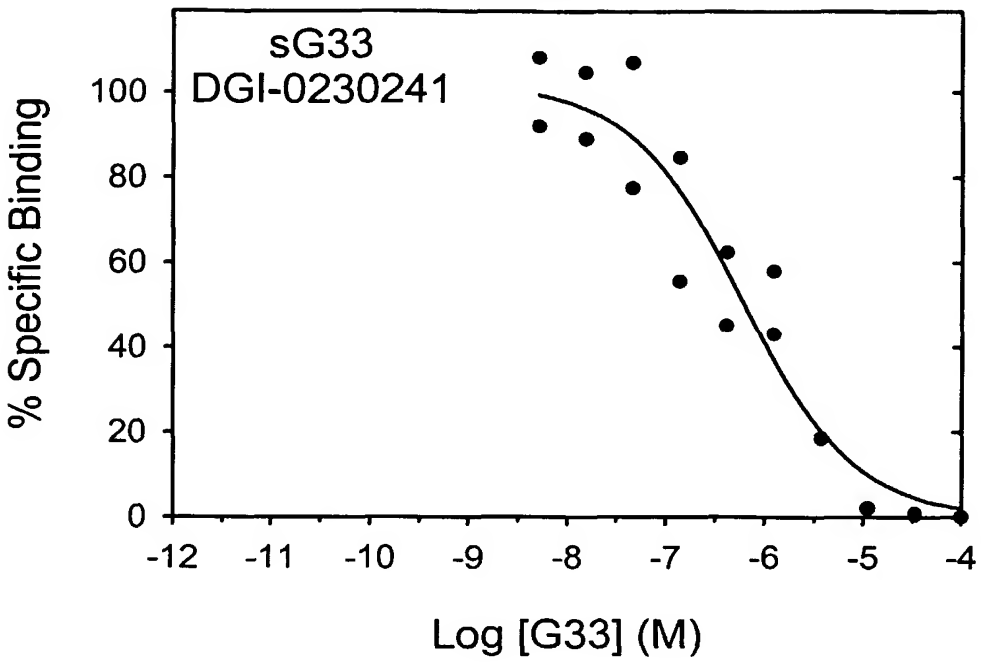


FIG. 71C

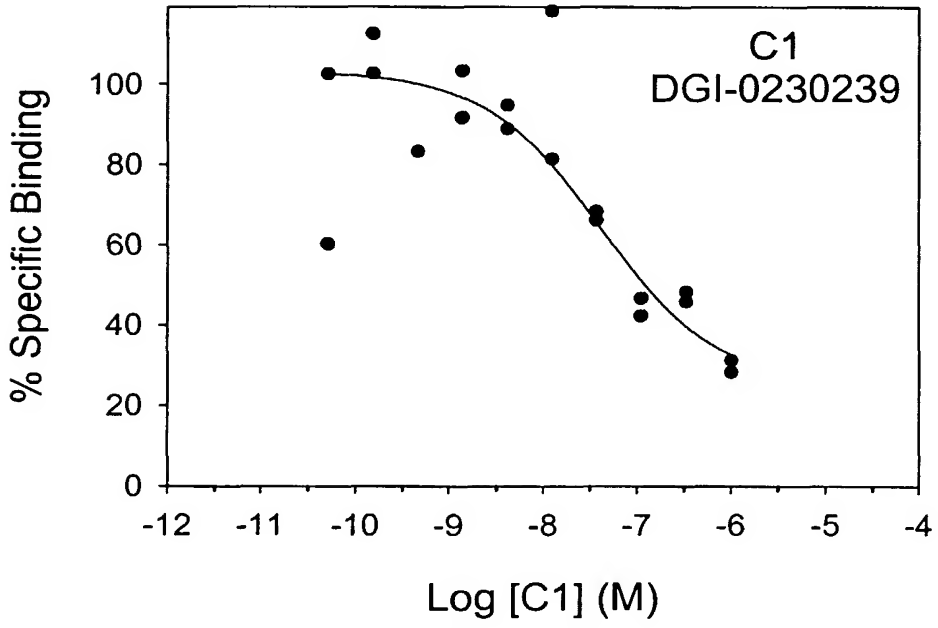


FIG. 71D

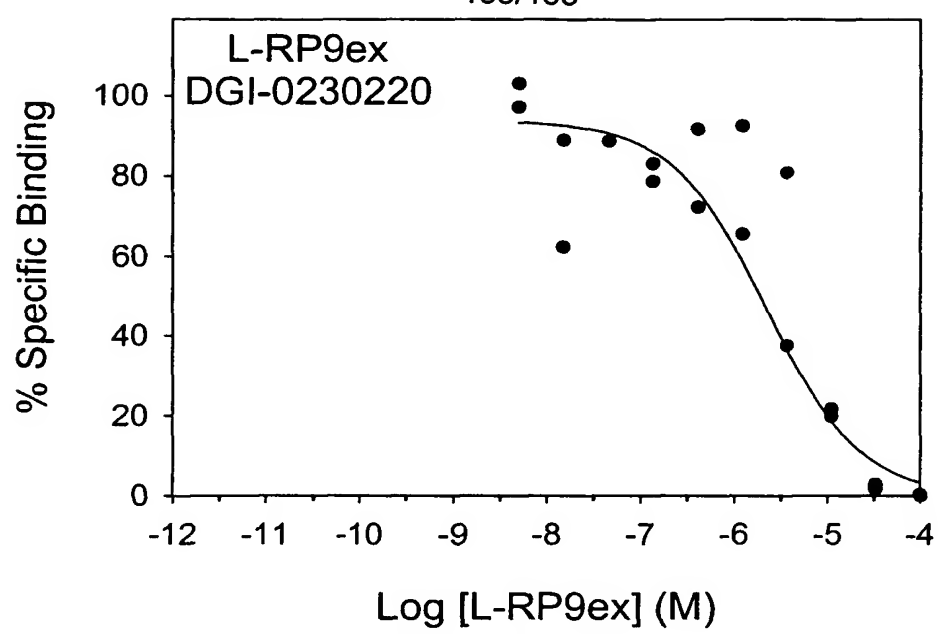


FIG. 71E

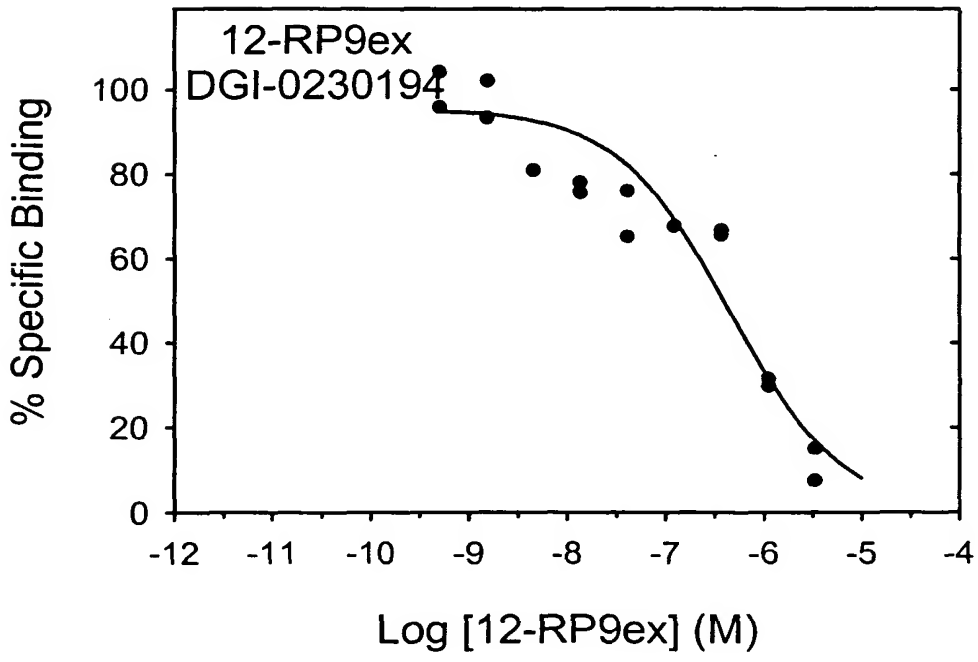


FIG. 71F

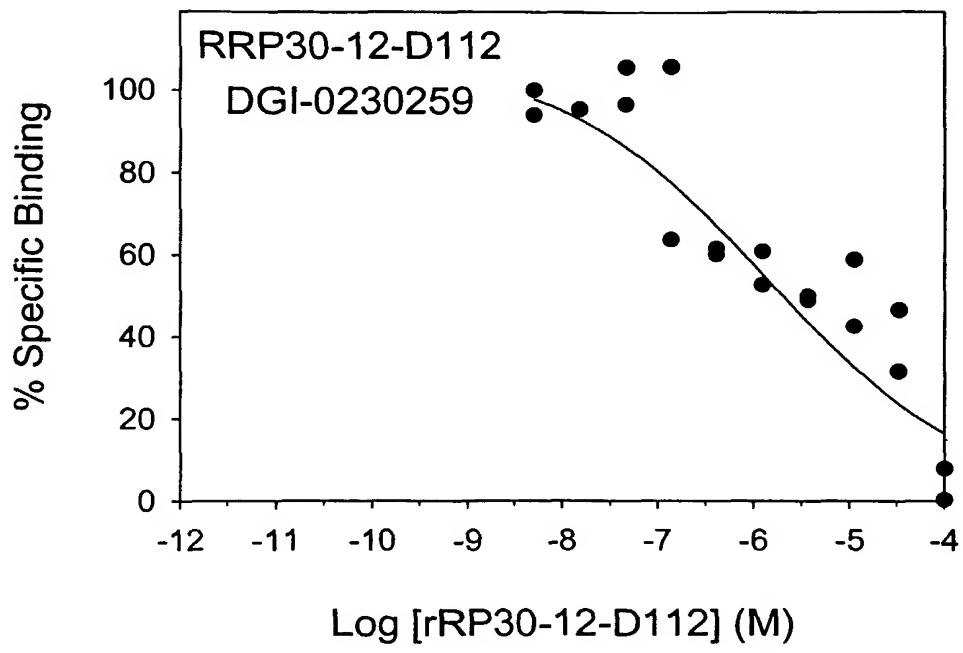


FIG. 72A

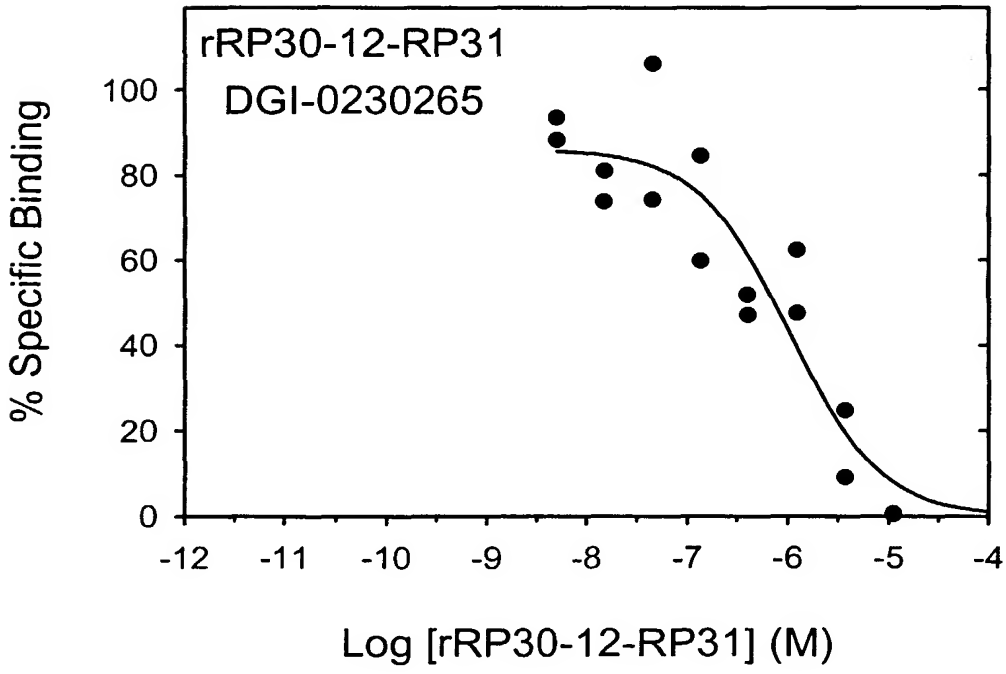


FIG. 72B

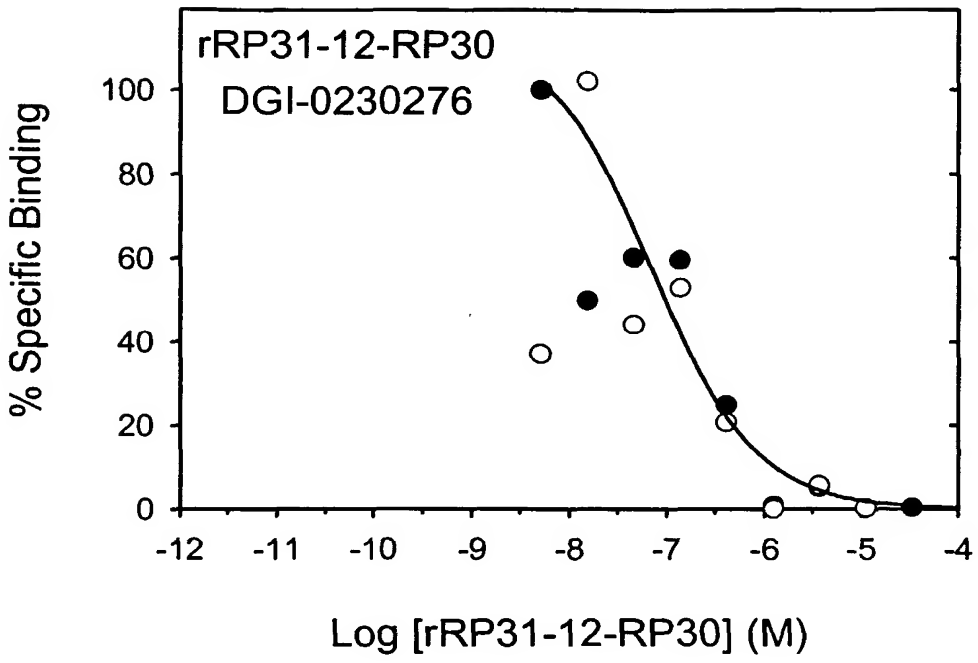


FIG. 72C

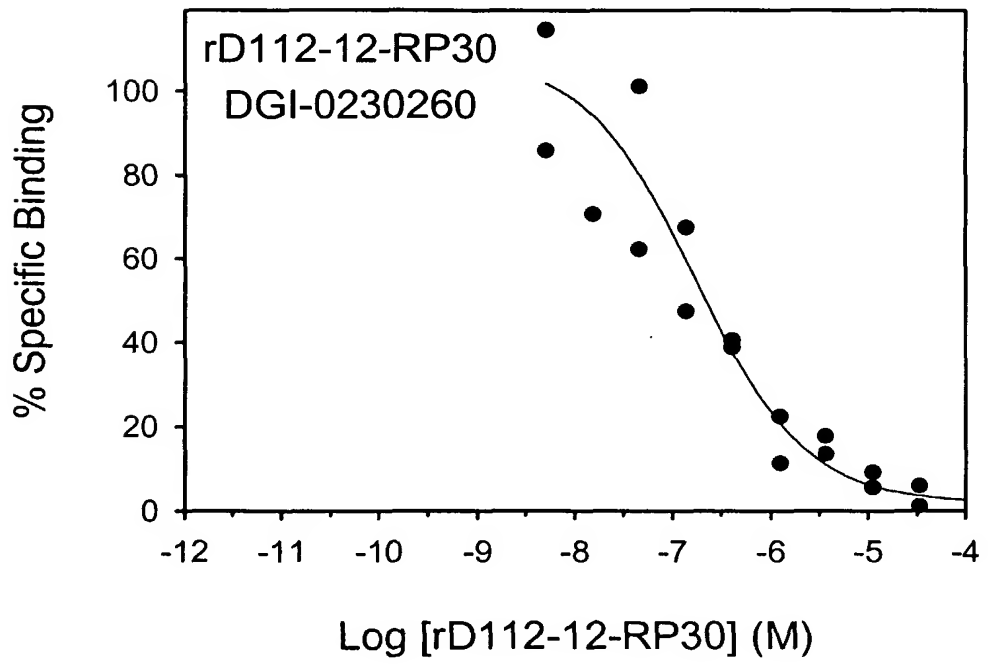


FIG. 72D

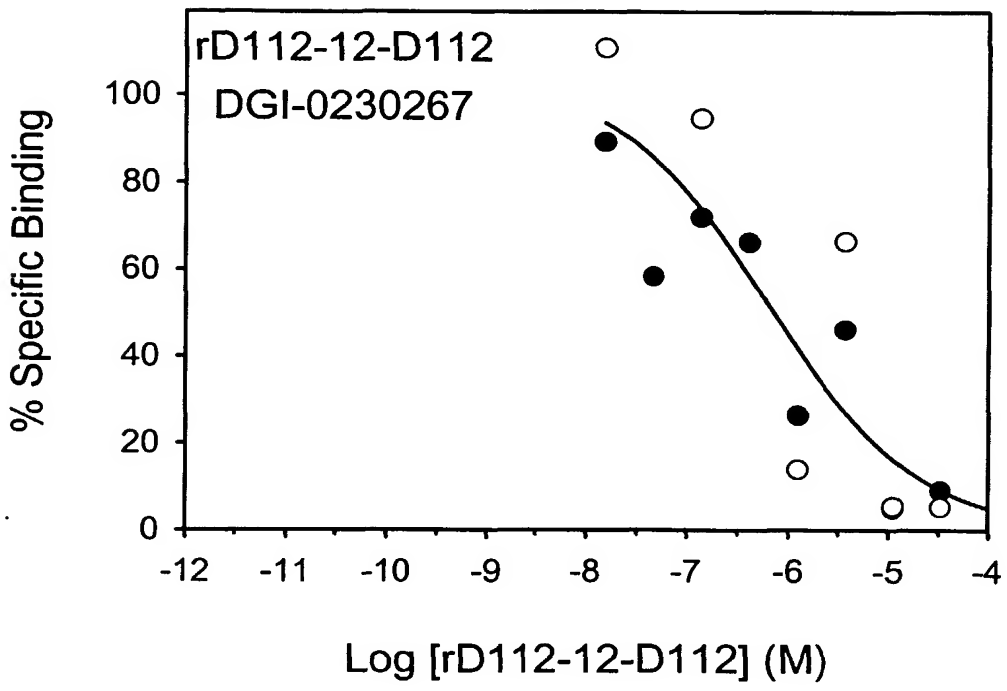

rD112-12-D112
DGI-0230267
% Specific Binding
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
-5
-4
Log [rD112-12-D112] (M)

FIG. 72E

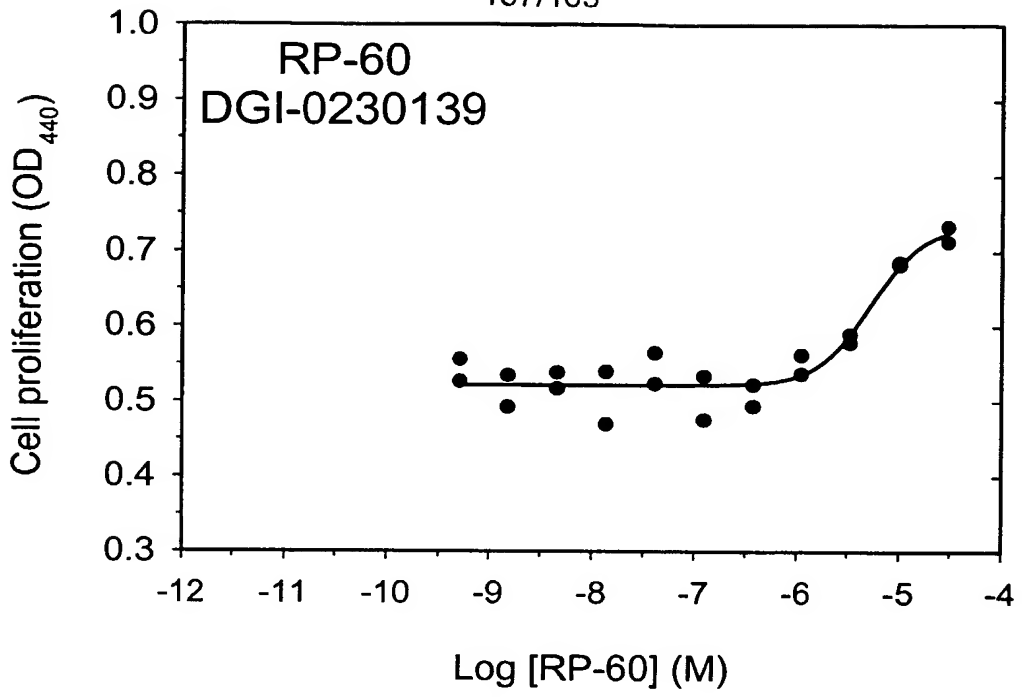


FIG. 73A

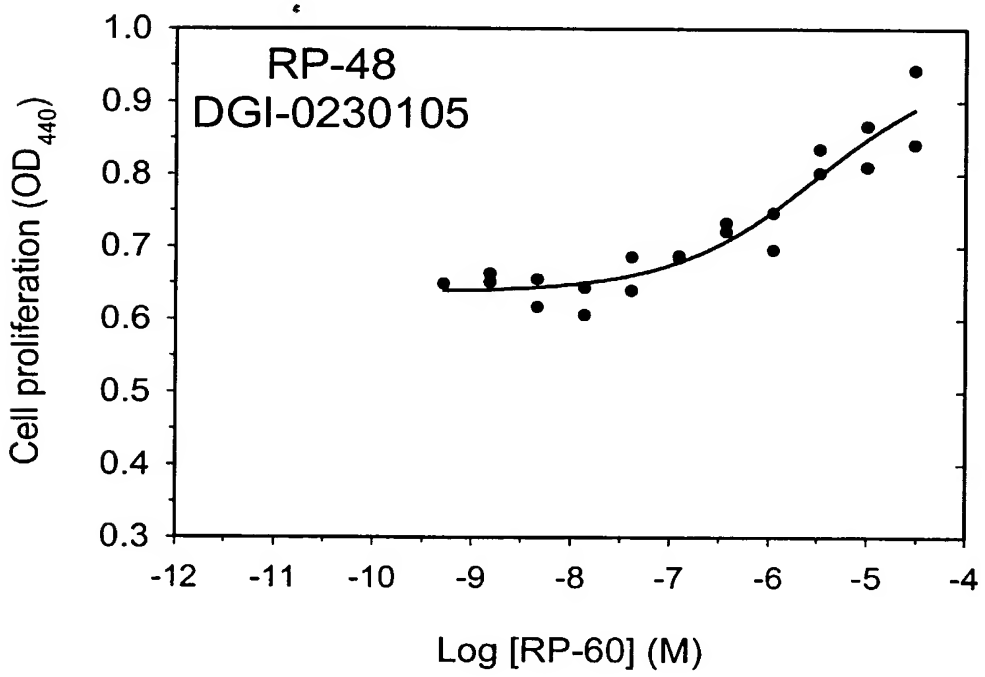


FIG. 73B

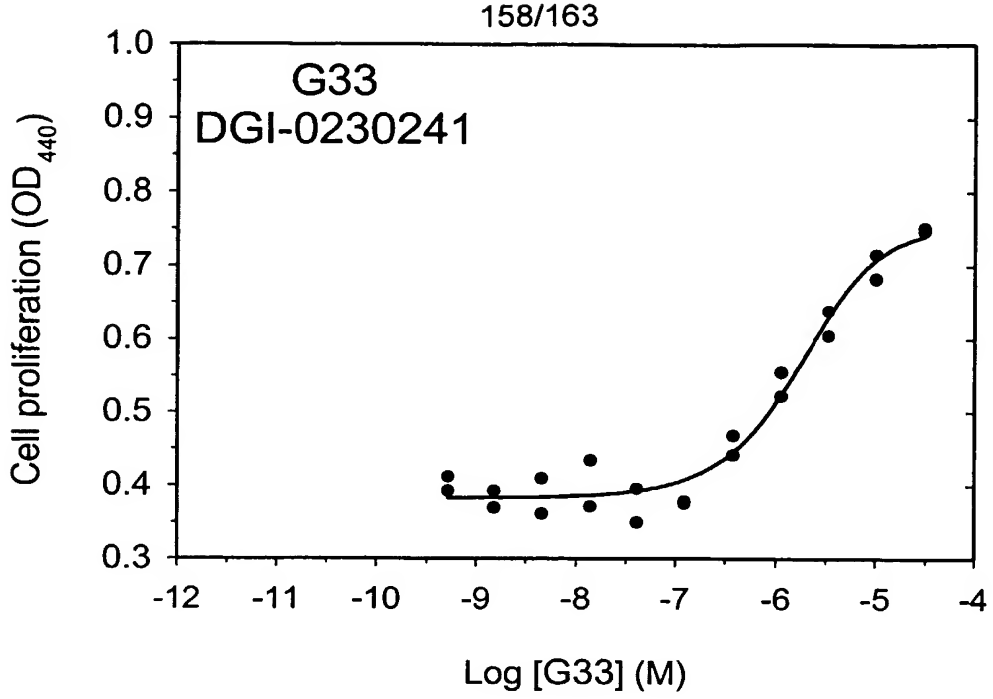


FIG. 73C

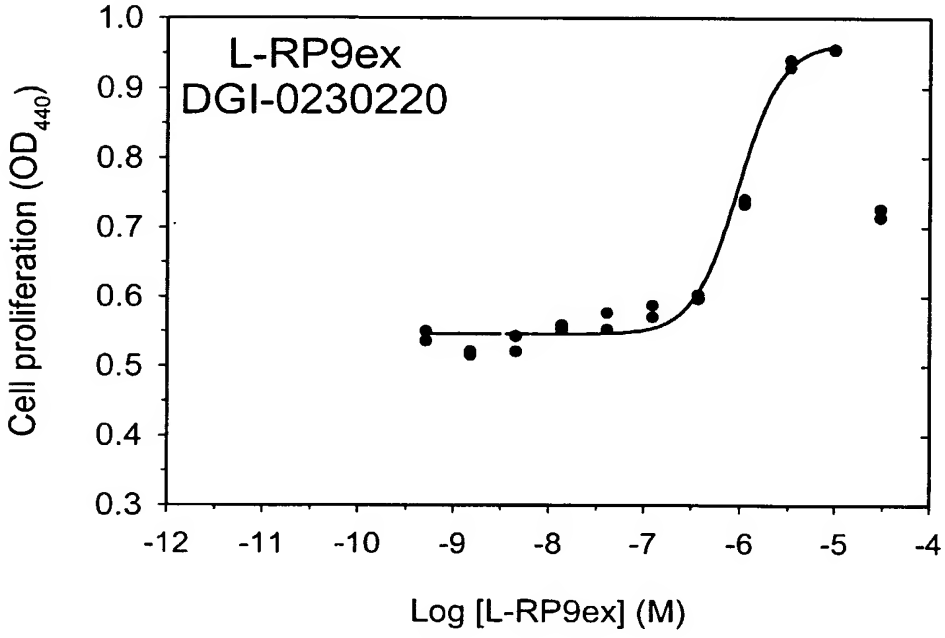


FIG. 73D

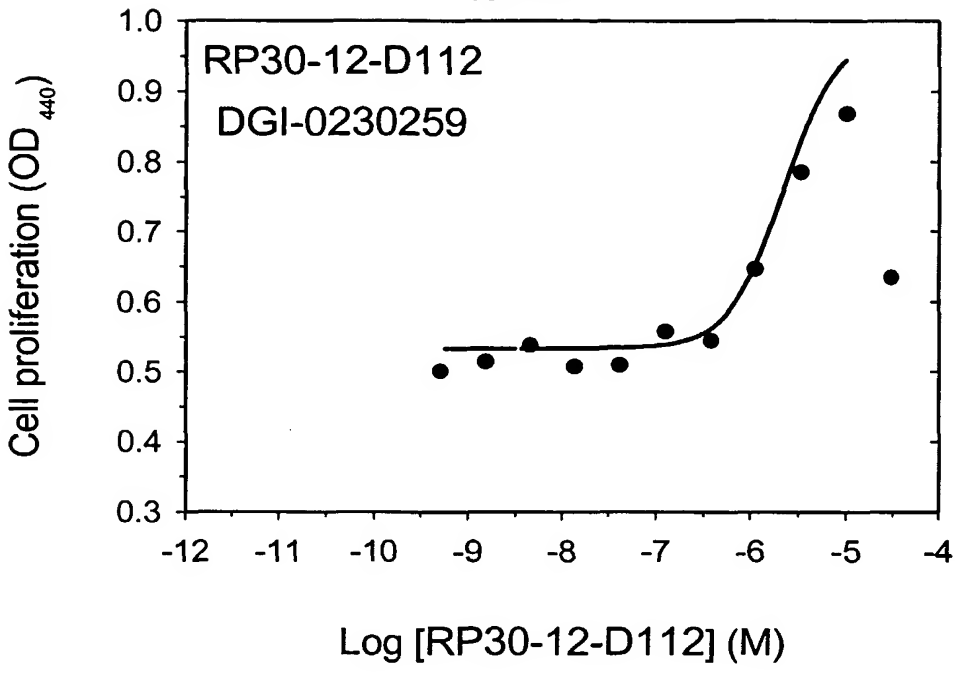


FIG. 74A

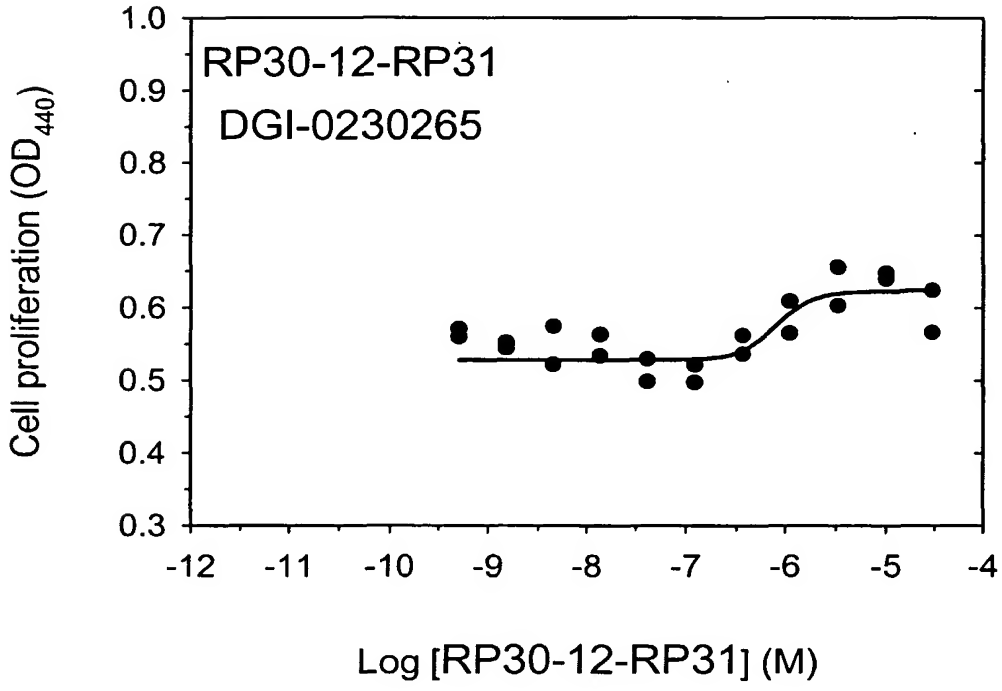


FIG. 74B

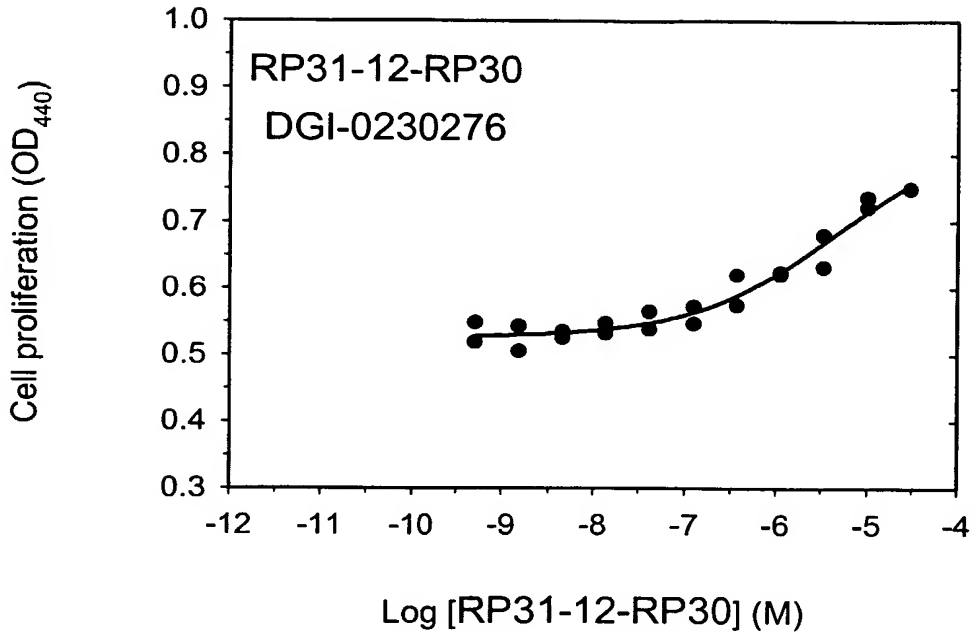


FIG. 74C

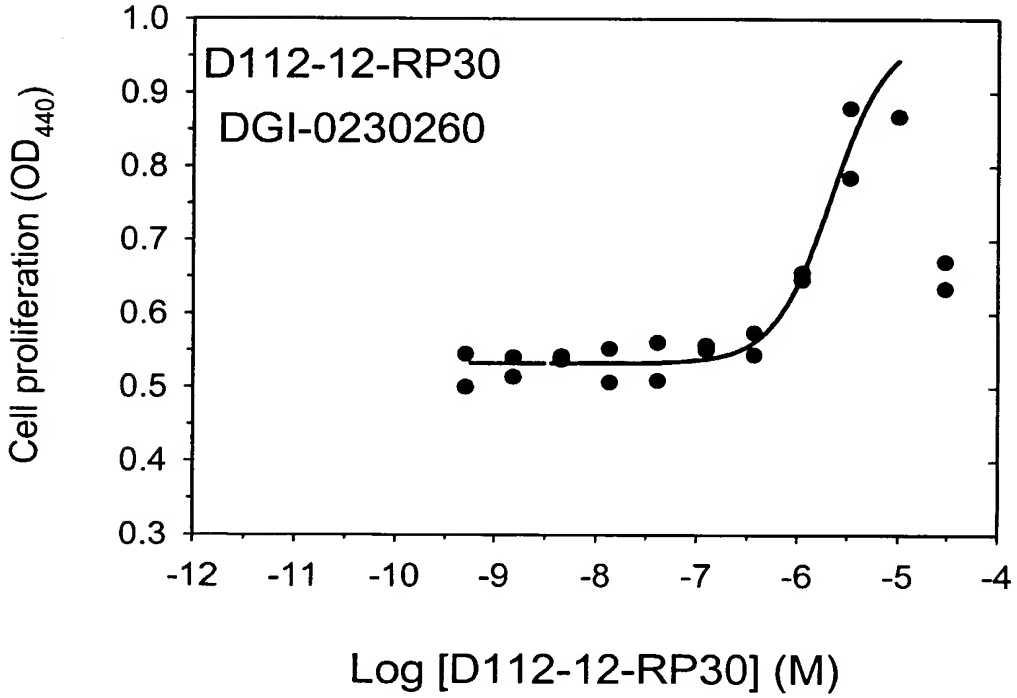


FIG. 74D

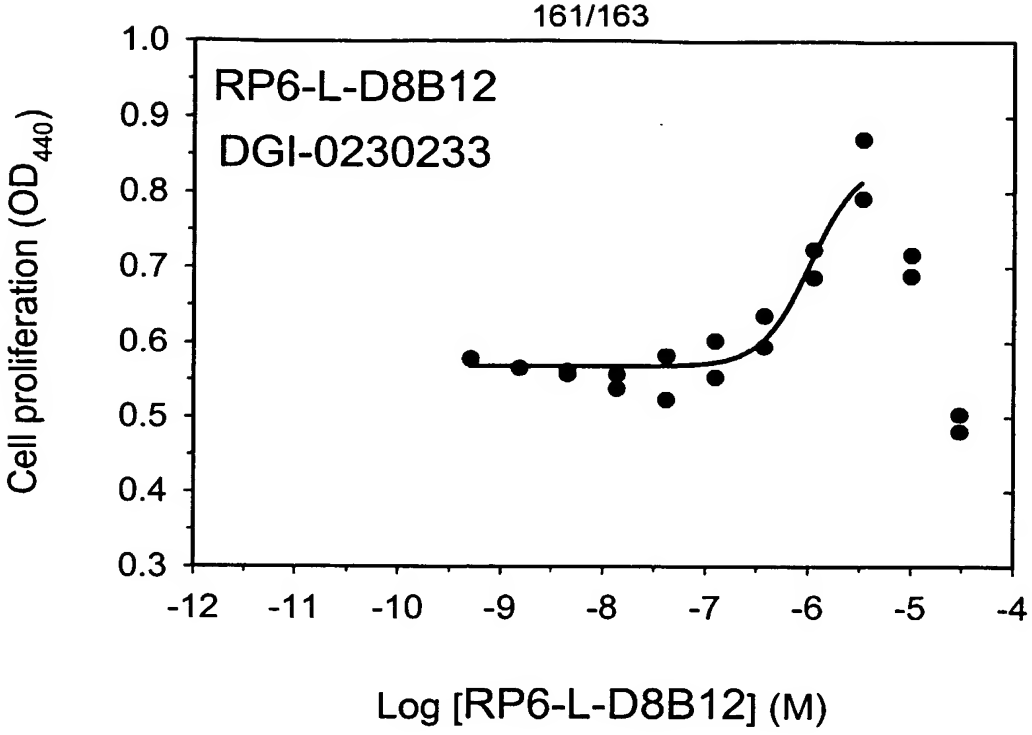


FIG. 74E

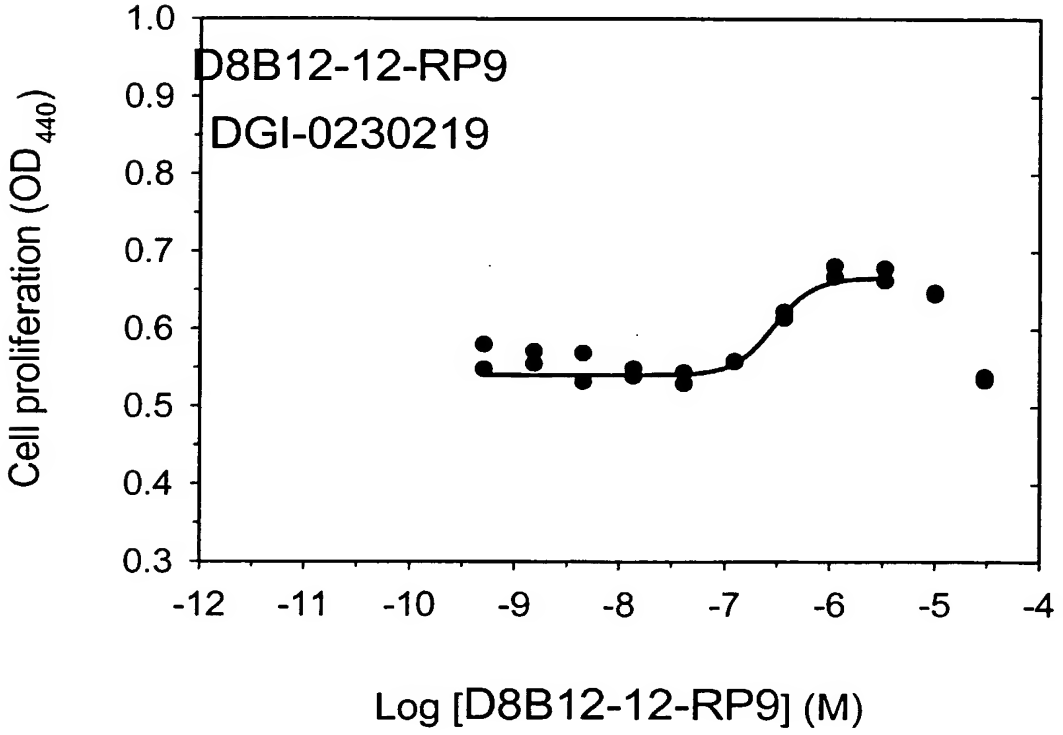


FIG. 74F

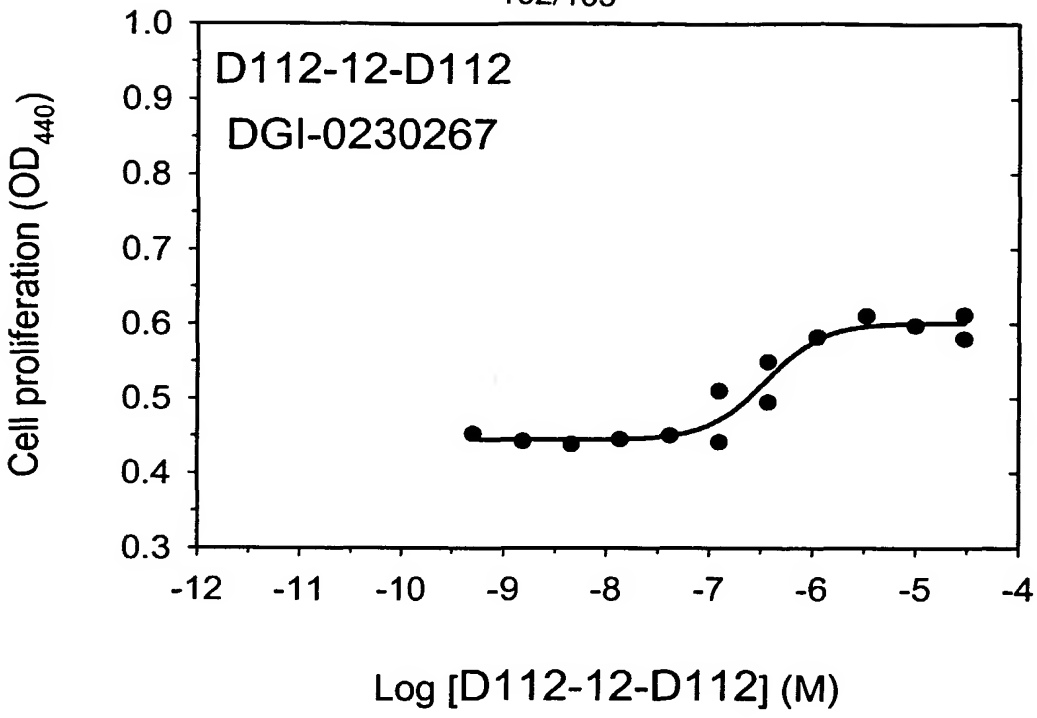


FIG. 74G

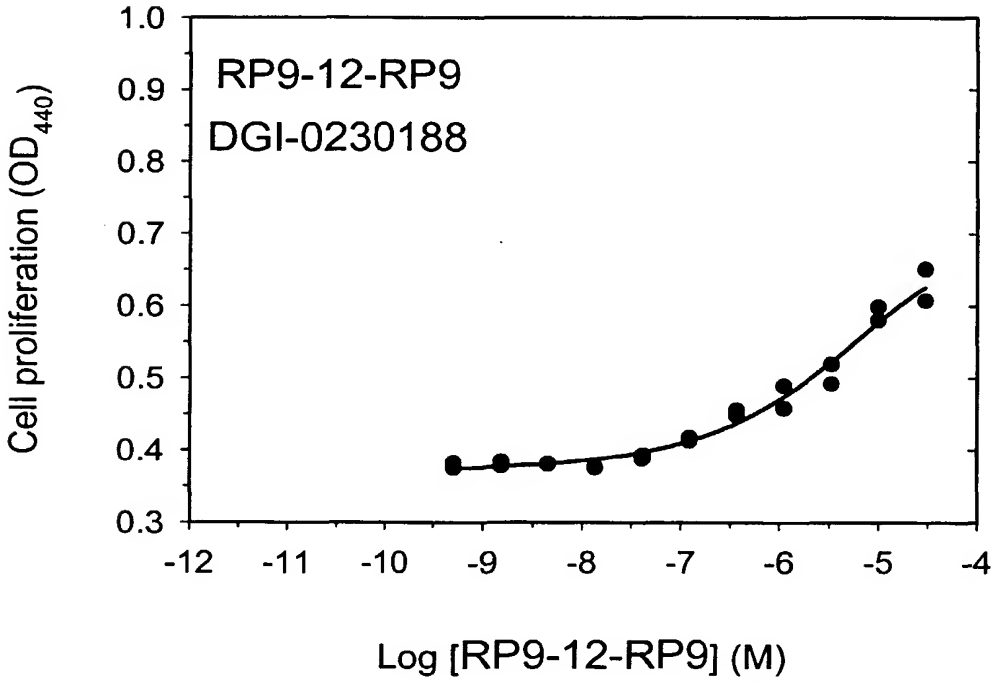


FIG. 74H

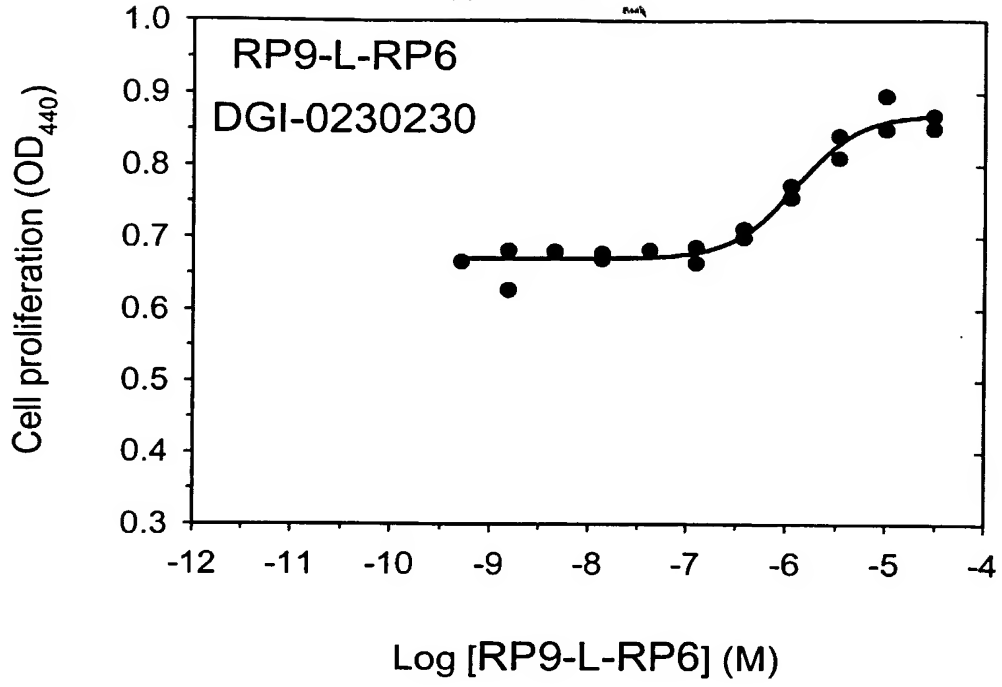

RP9-L-RP6
DGI-0230230
Cell proliferation (OD_{440})
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
-12
-11
-10
-9
-8
-7
-6
-5
-4
Log [RP9-L-RP6] (M)

FIG. 74I

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 April 2003 (03.04.2003)

PCT

(10) International Publication Number
WO 03/027246 A3

(51) International Patent Classification[7]: A61K 38/00, 38/08, G01N 33/567

(21) International Application Number: PCT/US02/30412

(22) International Filing Date:
24 September 2002 (24.09.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/962,756 24 September 2001 (24.09.2001) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 09/962,756 (CIP)
Filed on 24 September 2001 (24.09.2001)

(71) Applicants *(for all designated States except US)*: **NOVO NORDISK A/S** [DK/DK]; Novo Allé, DK-2880 Bagsvaerd (DK). **DGI BIOTECHNOLOGIES** [US/US]; 40 Talmadge Road, Edison, NJ 08818-0424 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PILLUTLA, Renuka** [IN/US]; 10 Pope Road, Bridgewater, NJ 08807 (US). **DEDOVA, Olga** [—/US]; 68 Forrest Glen Drive, Highland Park, NJ 08904 (US). **BLUME, Arthur, J.** [US/US]; 11 Walden Drive, Annandale, NJ 08801 (US). **GOLDSTEIN, Neil, I.** [US/US]; 26 Kendal Avenue, Maplewood, NJ 07040 (US). **BRISSETTE, Renee** [US/US]; 20 Deer Trail Drive, Clarksburg, NJ 08510 (US). **WANG, Pinger** [—/US]; 3109 Birchwood Ct., No. Brunswick, NJ 08902 (US). **LIU, Hao** [—/US]; 42G Franklin Green S., 1 JFK Boulevard, Somerset, NJ 08873 (US). **HSIAO, Ku-chuan** [—/US]; 451 Milano Drive, Easton, PA 18040 (US). **LENNICK, Michael** [—/US]; 2 Hills Top Drive, Annandale, NJ 08801 (US). **FLETCHER, Paul** [—/US]; 424 Rivercrest Drive, Piscataway, NJ 08854 (US).

(74) Agents: **SONNENFELD, Kenneth, H.** et al.; Morgan & Finnegan L.L.P., 345 Park Avenue, New York, NY 10154 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(88) Date of publication of the international search report:
31 July 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/027246 A3

(54) Title: ANTAGONISTS

(57) Abstract: Peptide sequences capable of binding to insulin and/or insulin-like growth factor receptors with either agonist or antagonist activity and identified from various peptide libraries are disclosed. This invention also identifies at least two different binding sites, which are present on insulin and insulin-like growth factor receptors, and which selectively bind the peptides of this invention. As agonists, the peptides of this invention may be useful for development as therapeutics to supplement or replace endogenous peptide hormones. The antagonist peptides may also be developed as therapeutics.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/30412

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/00, 38/08; G01N 33/567
US CL : 514/2,3,12,17; 435/7.1, 7.21, 7.8

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/2,3,12,17; 435/7.1, 7.21, 7.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE: file reg, caplus, embase, biosis, medline, wpids.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A/P	US 6,403,764 B1 (DUBAQUIE et al.) 11 June 2002(11.06.2002), see entire patent.	1-3,40-41,48,63-64
A/P	PILLUTLA, R.C. et al., "Peptides Identify the Critical Hotspots Involved in the Biological Activation of the Insulin Receptor", J. Biol. Chem. Vol. 277, No. 25, June 21, 2002, pages 22590-22594, see entire article.	1-3,40-41,48,63-64

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 March 2003 (28.03.2003)	22 APR 2003
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Bennett Celsa Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/30412

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 5,8,9,14,15,18,21,22,24,25,28,29,46,47,49,50,53,54,56,57,60,61,65,67 and 73
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
claims unsupported by disclosure and incomplete regarding placement of amino acids (e.g.cys) relative to other sequences.

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: Please See Continuation Sheet

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group 1, claim(s) 1-3(in part), drawn to 1st IGF receptor (IGF-R) modulation method (Id 2228).

Groups 2-4, claim(s) 1-4 (in part), drawn to 1st IGF-R modulation method (use of peptides/proteins comprising 3 different seq. id's , respectively).

Groups 5-8, claim(s) 6-7 (in part), drawn to 2nd IGF-R modulation method (comprising 4 different id's, respectively).

Groups 9-14, claim 10 (in part), drawn to 3rd IGF-R modulation method (comprising 6 different seq. Id's, respectively).

Groups 15-16, claim(s) 11-13 (in part), drawn to 4th IGF-R modulation method (comprising 2 different seq. Id's, respectively).

Groups 17-20, claim(s) 16-17 (in part), drawn to 5th IGF-R modulation method (comprising 4 different seq. Id's, respectively).

Groups 21-23, claim(s) 19-20 (in part), drawn to 6th IGF-R modulation method (comprising 3 different seq. Id's, respectively).

Group 24, claim 23, drawn to 7th IGF-R modulation method (comprising 1 seq. Id)

Groups 25-26, claim(s) 26-27 (in part), drawn to 8th IGF-R modulation method (comprising 2 different seq. Id's, respectively).

Groups 27-28, claim 30 (in part), drawn to 9th IGF-R modulation method (comprising 2 different seq. Id's, respectively.

Group 29, claim(s) 31-33 (in part), drawn to 10th IGF-R modulation method (comprising 1 seq. Id.).

Groups 30-31, claim(s) 34-37 (in part), drawn to 11th IGF-R modulation method (comprising 2 different seq. Id's, respectively).

Groups 32-53, claim(s) 38-39 (in part), drawn to 12th IGF-R modulation method (comprising 23 different seq. Id's, respectively).

Groups 54-74, claim(s) 40-41 (in part), drawn to 13th IGF-R modulation method (comprising 21 different seq. Id's, respectively).

Groups 75-78, claim(s) 42-45 (in part), drawn to 1st composition.

Groups 79-84, claim 48 (in part), drawn to composition comprising 6 different seq. Id's.

Groups 85-87, claim(s) 51-52 (in part), drawn to composition comprising 3 different seq. Id's.

Group 88, claim 55, drawn to 11th different composition.

Groups 89-90, claim(s) 58-59 (in part), drawn to 12th/13th different compositions comprising different seq. Id's.

Groups 91-92, claim(s) 62 (in part), drawn to 14th/15th different compositions comprising different seq. id's.

Groups 93-119, claim(s) 63-64 (in part), drawn to 27 different compositions comprising different seq. Id's.

Groups 120-123, claim(s) 66 (in part), drawn to 1st library screening method (comprising use of peptides/proteins comprising 4 different seq. Id's).

Groups 124-129, claim(s) 68 (in part), drawn to 2nd library screening method (comprising use of peptides/proteins comprising 6 different seq. Id's).

Groups 130-131, claim(s) 69-70 (in part), drawn to 3rd library screening method (comprising use of peptides/proteins comprising 2 different seq. Id's).

Groups 132-153, claim(s) 71 (in part), drawn to 4th library screening method (comprising use of peptides/proteins comprising 22 different seq. Id's).

Groups 154-175, claim(s) 72 (in part), drawn to 5th library screening method (comprising use of peptides/proteins comprising 21 different seq. Id's).

Group 176, claim (s) 74 (in part), drawn to 1st cell survival method (using formula 1 peptides/proteins).

Group 177 , claim(s) 74 (in part), drawn to 2nd cell survival method (using formula 2 peptides/proteins).

Group 178, claim(s) 74 (in part), drawn to 3rd cell survival method (using formula 3 peptides/proteins). The inventions listed as Groups 1-178 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the claimed methods.address different method objectives and/or utilize structurally distinct peptides/proteins which are not coextensive and which do not share the same technical feature. The peptides/protein compositions of the different groups are unrelated and/or structurally distinct (e.g. different amino acid composition and length) and do not share the same technical feature.

Note: Above lack of unity subject PCT Article 14(4) [e.g. limited search facilities] and PCT Article 17(1)(ii) [e.g. extend disclosure/claims/drawings limit a meaningful search].

Unsearchable Claims: Claims 5, 8-9, 14-15, 18, 21-22, 24, 25, 28, 29, 46, 47, 49, 53, 54, 56, 57, 60, 61, 65, 67 and 73.

Continuation of Box II Item 3:
1-3,40-41,48,63-64 (in part:id 2228,2232,2234,2236,2244,2249,2253,2255,2261,2262)